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(71) Applicant (for all designated States except US):
CXR BIOSCIENCES LIMITED [GB/GB]; Dundee
Technopole, James Lindsay Place, Dundee DD1 5JJ (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ELCOMBE, Clif-
ford, Roy** [GB/GB]; CXR Biosciences Limited, James
Lindsay Place, Dundee DD1 5JJ (GB). **WOLF, Charles,
Roland** [GB/GB]; CXR Biosciences Limited, James
Lindsay Place, Dundee DD1 5JJ (GB).

(74) Agent: **MILES, John, S.**; Eric Potter Clarkson, Park View
House, 58 The Ropewalk, Nottingham NG1 5DD (GB).

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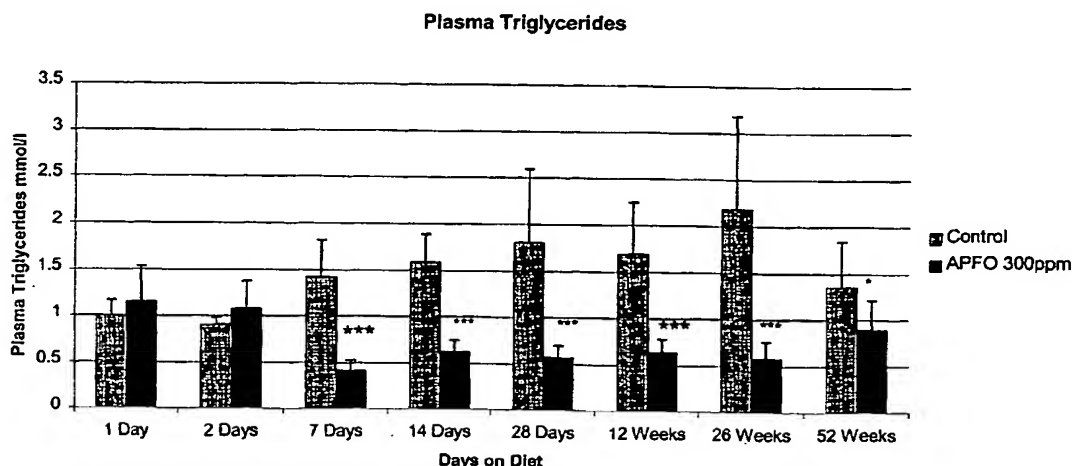
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(57) Abstract: The molecules of formula (I) are useful in treating diabetes, obesity, hypercholesterolaemia, hyperlipidaemia, cancer, inflammation or other conditions in which modulation of lipid or eicosanoid status or functions may be desirable. Formula (I): Z^1-X-Z^2 wherein a) Z^1 represents CO_2H or a derivative thereof; b) Z^2 represents F, H, $-CO_2H$ or a derivative thereof; and c) X represents fluorinated alkylene; or a solvate thereof, for example a perfluorinated fatty acid or derivative thereof.

METHODS

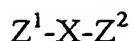
This invention relates to the medical use of compounds, and to methods of identifying further useful compounds, particularly in the treatment of diabetes, obesity, hyperlipidaemia, hypercholesterolaemia, atherosclerosis, cancer and inflammation, or other conditions where alterations in lipid or eicosanoid status may be desirable.

Perfluorooctanoic acid (PFOA) and other perfluorinated fatty acids or fluoroalkyl molecules are synthetic molecules used in industrial applications, principally as surfactants. The effects of these compounds on laboratory animals and cells has been studied, as have the effects of occupational exposure in humans (see, for example, Gilliland & Mandel (1993) *J Occup Med* 35(9), 950-954; Kees *et al* (1992) *J Med Chem* 35, 944-953). US 4,624,851 suggests treatment of symptoms of cancer using fluorine containing acids; no experimental data is presented.

We have surprisingly found that such compounds may have beneficial effects. We have found that such compounds may be useful in treatment of diabetes, obesity, hyperlipidaemia, hypercholesterolaemia, atherosclerosis, cancer and inflammation, or other conditions where alterations in lipid or eicosanoid status may be desirable.

A first aspect of the invention provides a method of treatment of a patient in need of modulation (preferably reduction) of body mass or modulation (preferably prevention or reduction) of increase in body mass, and/or in need of modulation (preferably reduction) of plasma insulin, plasma glucose, plasma triglycerides and/or plasma cholesterol, comprising administering to the patient an effective amount of a compound of formula I as defined herein.

The compound of formula I is



I

wherein

Z^1 represents $-CO_2H$ or a derivative thereof;

Z^2 represents F, H, $-CO_2H$ or a derivative thereof; and

5 X represents fluorinated alkylene;

or a solvate thereof;

which compounds are referred to hereinafter as "the compounds of the invention".

10 A further aspect of the invention provides a method of treatment of a patient in need of an antitumour agent or an antiinflammatory agent, or in need of modulation in lipid or eicosanoid status, comprising administering to the patient an effective amount of a compound of formula I as defined herein. A compound of formula I is considered to be effective as an antitumour agent or
15 an antiinflammatory agent or in modulating lipid or eicosanoid status (ie type and concentration of lipid or eicosanoid, either systemically or in a particular locus or tissue).

The patient may be a patient with or at risk of excessive inflammation, for
20 example with or at risk of arthritis, or a patient with or at risk of developing a tumour. The compound may reduce the development, growth or metastasis of a tumour.

The compound may be useful in treating any condition or disorder in which the
25 patient has or is at risk of excessive inflammation. The patient may have an allergic or autoimmune disease. The patient may have, for example, psoriasis, inflammatory bowel disease, asthma or rheumatism.

A further aspect of the invention provides a method of treatment of a patient
30 who is overweight or obese and/or has diabetes, hyperlipidaemia,

atherosclerosis, coronary heart disease, stroke, obstructive sleep apnoea, arthritis (for example osteoarthritis) and/or reduced fertility, or is at risk of developing such a condition, comprising administering to the patient an effective amount of a compound of formula I as defined herein.

5

A further aspect of the invention provides a method of treatment of a patient in need of modulation of PPAR (for example PPAR α , δ or γ) activity, comprising administering to the patient an effective amount of a compound of formula I as defined herein. The compound may be a PPAR agonist or a PPAR antagonist;
10 it may be an agonist for one PPAR and an antagonist for a different PPAR. Preferably the patient is in need of an increase in PPAR α or PPAR γ activity and the compound is a PPAR α or PPAR γ agonist. Alternatively, the patient may be in need of a decrease in PPAR α or PPAR γ activity and the compound may be a PPAR α or PPAR γ antagonist. In a further alternative, the patient
15 may be in need of an increase in PPAR δ activity and the compound is a PPAR δ agonist. In a still further alternative, the patient may be in need of a decrease in PPAR δ activity and the compound may be a PPAR δ antagonist. PPAR δ may have opposing effects to PPAR α or PPAR γ (see, for example, WO01/07066).

20 A further aspect of the invention provides a method of treatment of a patient in need of modulation of lipid or eicosanoid status or function, for example in need of modulation of the activity of a lipid metabolising or binding entity (including a lipid metabolising enzyme and a lipid binding polypeptide, for example a lipid transporting polypeptide), for example cyclooxygenase (for
25 example cyclooxygenase I or cyclooxygenase II) activity or phospholipase A (for example phospholipase A2) or lipoxygenase, comprising administering to the patient an effective amount of a compound of formula I as defined herein. Preferably the patient is in need of a decrease in a lipid metabolising or binding activity, for example cyclooxygenase (for example cyclooxygenase I or

cyclooxygenase II) activity or phospholipase A or lipoxygenase and the compound is an inhibitor of such activity. For example, inappropriate lipoxygenase activity may be involved in inflammation, hypersensitivity, asthma and some vascular diseases; thus a decrease in a lipoxygenase activity
5 may be useful in such a condition.

Alternatively, the patient may be in need of an increase in such activity and the compound may be an activator of such activity.

10 Further preferences in relation to the patient and compound are indicated below.

Compounds of formula I may exhibit tautomerism. All tautomeric forms and mixtures thereof are included within the scope of the invention.

15

Compounds of formula I may also contain one or more asymmetric carbon atoms and may therefore exhibit optical and/or diastereoisomerism. Diastereoisomers may be separated using conventional techniques, e.g. chromatography or fractional crystallisation. The various stereoisomers may
20 be isolated by separation of a racemic or other mixture of the compounds using conventional, e.g. fractional crystallisation or HPLC, techniques. Alternatively the desired optical isomers may be made by reaction of the appropriate optically active starting materials under conditions which will not cause racemisation or epimerisation, or by derivatisation, for example with a
25 homochiral acid followed by separation of the diastereomeric esters by conventional means (e.g. HPLC, chromatography over silica). All stereoisomers are included within the scope of the invention.

When referred to herein, derivatives of $-CO_2H$ groups include groups which are
30 commonly derived from a carboxylic acid and/or groups that contain a central

carbon atom (i.e. the carbon atom that is attached to X) that is at the same oxidation state as -C(O)OH . Derivatives of $\text{-CO}_2\text{H}$ groups therefore includes groups such as:

- (i) esters, e.g. those formed with an alcohol of formula R^1OH , wherein R^1 represents aryl or alkyl;
- (ii) thioesters, e.g. those formed with a thiol of formula R^1SH , wherein R^1 is as hereinbefore defined; and
- (iii) salts, e.g. those formed with a nitrogen-containing base such as ammonia, an alkylamine, a dialkylamine, a trialkylamine and pyridine or alkali or alkaline earth metal salts (e.g. Na, K, Cs, Mg or Ca salts).

Preferred derivatives of $\text{-CO}_2\text{H}$ groups include those that are pharmaceutically acceptable.

Where the term fluorine is used herein, it is intended (where appropriate) that reference to other halogens, for example chlorine or bromine or more than one halogen, is included. However, it is strongly preferred that the halogen is fluorine.

It is preferred that the compound of Formula I comprises at least two fluorine atoms, preferably at least three, four, five, six, seven or eight fluorine atoms.

The term "aryl", when used herein, includes C_{6-10} aryl groups such as phenyl, naphthyl and the like. Aryl groups may be substituted by one or more substituents including -OH , cyano, halo, nitro, amino, alkyl and alkoxy. When substituted, aryl groups are preferably substituted by between one and three substituents.

The term alkyl, when used herein, refers to alkyl groups of 1 to 16, preferably 1 to 10 (e.g. 1 to 6) carbon atoms.

The term alkoxy, when used herein, refers to alkoxy groups of 1 to 16, preferably 1 to 10 (e.g. 1 to 6) carbon atoms.

- 5 Alkyl and alkoxy groups as defined herein may be straight-chain or, when there is a sufficient number (i.e. a minimum of three) of carbon atoms, be branched-chain and/or cyclic and/or heterocyclic. Further, when there is a sufficient number (i.e. a minimum of four) of carbon atoms, such alkyl and alkoxy groups may also be part cyclic/acyclic. Such alkyl and alkoxy groups may also be
- 10 saturated or, when there is a sufficient number (i.e. a minimum of two) of carbon atoms, be unsaturated and/or interrupted by one or more oxygen and/or sulfur atoms. Alkyl and alkoxy groups may also be substituted by one or more halo, and especially fluoro, atoms.
- 15 The term "halo", when used herein, includes fluoro, chloro, bromo and iodo.

The terms alkylamine, dialkylamine and trialkylamine, when used herein, refer to amines bearing one, two or three alkyl groups as defined herein, respectively.

20

- The term alkylene, when used herein, refers to alkylene groups of 1 to 20, preferably 2 to 17 (e.g. 6 to 12) carbon atoms. Alkylene groups may be straight-chain or, when there is a sufficient number (i.e. a minimum of two or three, as appropriate) of carbon atoms, be branched-chain and/or cyclic and/or
- 25 heterocyclic. Further, when there is a sufficient number (i.e. a minimum of four) of carbon atoms, such alkylene groups may also be part cyclic/acyclic. Such alkylene chains may also be saturated or, when there is a sufficient number (i.e. a minimum of two) of carbon atoms, be unsaturated and/or interrupted by one or more oxygen and/or sulfur atoms.

30

Preferred compounds of formula I include those in which:

alkylene group X is at least 50% fluorinated;

alkylene group X contains between 2 and 17 carbon atoms;

Z^1 represents $-\text{CO}_2\text{H}$, an ammonium, $(\text{C}_{1-10}$ alkyl)ammonium, di- $(\text{C}_{1-10}$

5 alkyl)ammonium or tri- $(\text{C}_{1-10}$ alkyl)ammonium salt of $-\text{CO}_2\text{H}$, or $-\text{CO}_2\text{R}^1$;

Z^2 represents F, H, $-\text{CO}_2\text{H}$ or $-\text{CO}_2\text{R}^1$;

R^1 represents C_{1-6} alkyl.

More preferred compounds of formula I include those in which:

10 alkylene group X is at least 75% fluorinated;

alkylene group X is straight-chain, saturated and contains between 4 and 14 carbon atoms;

Z^1 represents $-\text{CO}_2\text{H}$, an ammonium, $(\text{C}_{1-6}$ alkyl)ammonium, di- $(\text{C}_{1-6}$ alkyl)ammonium or tri- $(\text{C}_{1-6}$ alkyl)ammonium salt of $-\text{CO}_2\text{H}$, or $-\text{CO}_2\text{R}^1$;

15 Z^2 represents F, $-\text{CO}_2\text{H}$ or $-\text{CO}_2\text{R}^1$;

R^1 represents straight-chain, unsubstituted, saturated C_{1-4} alkyl.

Even more preferred compounds of formula I include those in which:

alkylene group X is at least 90% fluorinated;

20 alkylene group X is straight-chain, saturated and contains between 6 and 12 carbon atoms;

Z^1 represents $-\text{CO}_2\text{H}$, an ammonium salt of $-\text{CO}_2\text{H}$, or $-\text{CO}_2\text{R}^1$;

R^1 represents straight-chain, unsubstituted, saturated C_{1-2} alkyl.

25 Particularly preferred compounds of formula I may be or comprise a member of the following group:

Perfluoroheptanoic acid; perfluorooctanoic acid; perfluorononanoic acid;

perfluorodecanoic acid; perfluoroundecanoic acid; perfluorododecanoic acid;

perfluorotetradecanoic acid; perfluorohexadecanoic acid;

30 perfluorooctadecanoic acid;

perfluorosuccinic acid; perfluoroglutaric acid; perfluoroadipic acid;
perfluorosuberic acid; perfluoroazelaic acid; perfluorosebacic acid; perfluoro-
1, 10-decanedicarboxylic acid;
methyl perfluoroheptanoate; methyl perfluorooctanoate; methyl
5 perfluorononanoate; methyl perfluorodecanoate; methyl perfluoroundecanoate;
methyl perfluorododecanoate; methyl perfluorotridecanoate; methyl
perfluorotetradecanoate; methyl perfluoropentadecanoate; methyl
perfluorohexadecanoate; methyl perfluorooctadecanoate;
dimethyl perfluorosuccinate; dimethyl perfluoroglutarate; dimethyl
10 perfluoroadipate; dimethyl perfluorosuberate; dimethyl perfluoroazelate;
dimethyl perfluorosebacate; perfluoro- 1, 10-decanedicarboxylic acid, dimethyl
ester; and dimethyl perfluorododecanedioate.

These compounds may be obtained from any suitable supplier, for example
15 3M, DuPont, Miteni or Dyneon.

Examples of fluoroalkyl carbonyl compounds that may be useful include alpha-
branched fluoroalkylcarbonyl fluorides and derivatives thereof, as described in
US 6, 013, 795 or US 6,015,838 (both incorporated herein by reference) and
20 references given therein, for example US 2,567,011 (incorporated herein by
reference). Methods of preparing same are also described. These compounds
may also be useful in the synthesis of further compounds of the invention.

It is preferred that the compound is not a compound as discussed in US
25 6,028,109, which are indicated to be PPAR agonists. Thus, it is preferred that
the compound is not a compound represented by formula (I) of US 6,028,109
(shown in Figure 8).

Particularly preferred compounds of formula I include fluorinated fatty acids,
30 such as perfluorinated fatty acids, for example perfluorooctanoic acid (PFOA)

or a derivative or pharmaceutically acceptable salt or ester thereof (e.g. ammonium perfluorooctanoate (APFO)). The chemical formula for APFO is $\text{CF}_3(\text{CF}_2)_6\text{COO}^- \text{NH}_4^+$ (octanoic acid, pentadecafluoro-, ammonium salt; C-8, FC-143; CAS Registry No 3825-26-1). It may be obtained from DuPont
5 (DuPont Chemical Solutions Enterprise, DuPont-Strassel, D-61343 Bad Homburg, Germany). Common contaminants of APFO include ammonium perfluoroheptanoate (CAS 6130-43-4), ammonium perfluorohexanoate (CAS 68259-11-0), ammonium perfluoropentanoate (CAS 21615-47-4), and branched chain homologs that are generically known as ammonium
10 perfluoroisooctanoate, ammonium perfluoroisoheptanoate, ammonium perfluoroisohexanoate and ammonium perfluoroisopentanoate. Whilst it is considered that the effects observed in Example 1 using an APFO preparation arise from the administration of APFO itself, it will be appreciated that one or more contaminants, for example one or more of the possible contaminants
15 listed above, may contribute to the effects observed.

It is preferred that the compound is not PFOS (perfluorooctylsulphonate) or perfluorodecanoic acid or a derivative or salt or ester thereof; these compounds may have toxic or environmentally undesirable effects.

20

It is preferred that the compound of formula I (or identified or identifiable by a screening method of the invention, as discussed below) is metabolically stable; for example it is preferred that the compound has a similar rate of metabolism to perfluorooctanoic acid. The compound may be considered to be a lipid
25 mimetic which may be metabolically stable.

A further aspect of the invention provides the use of a compound of formula I as defined herein in the manufacture of a medicament for the treatment of a patient in need of modulation (preferably reduction) of body mass or
30 modulation (preferably prevention or reduction) of increase in body mass,

and/or in need of modulation (preferably reduction) of plasma insulin, plasma glucose, plasma triglycerides, leptin and/or plasma cholesterol. The patient may (for example in relation to a decrease in the above-listed parameters) be overweight or obese and/or have diabetes, hyperlipidaemia and/or
5 atherosclerosis, or be at risk of developing such a condition. The risk may arise from genetic factors, age, or environmental factors, such as diet.

The patient may have other condition(s) associated with obesity, for example coronary heart disease, stroke, obstructive sleep apnoea, arthritis (for example
10 osteoarthritis) or reduced fertility.

Accordingly, a further aspect of the invention provides the use of a compound of formula I as defined herein in the manufacture of a medicament for the treatment of a patient who is overweight or obese and/or has diabetes,
15 hyperlipidaemia, atherosclerosis, coronary heart disease, stroke, obstructive sleep apnoea, arthritis (for example osteoarthritis) and/or reduced fertility, or is at risk of developing such a condition.

A further aspect of the invention provides the use of a compound of formula I
20 in the manufacture of a medicament for the treatment of a patient in need an antitumour agent or an antiinflammatory agent or of modulation of lipid or eicosanoid status. Preferences in relation to such a patient are noted above.

As is well known to those skilled in the art, obesity may be described as a state
25 of excessive accumulation of body fat. Obesity may be determined by determining the body mass index (BMI) for a patient, and/or by measuring subcutaneous fat deposits in the arm using a "pinch test". The BMI is defined as weight (in kilograms) divided by the square of the height in metres. A BMI of 25-30 is considered as overweight and more than 30 as obese. Preferably,

treatment leads to lowering of the BMI to less than about 29 to 31, or to a point at which health risks from being overweight are no longer significant.

It will be appreciated that the treatment of the invention may be used in
5 combination with other treatments for the relevant condition. For example in relation to obesity, the patient may follow a calorie-restricted diet and/or follow a program of physical exercise.

The medicament may comprise more than one (e.g. two) compounds of
10 formula I (such as perfluorinated fatty acids or their derivatives). The medicament may comprise a prodrug, for example a molecule which is converted to a molecule with the required biological activity following administration of the medicament to the patient.

15 Compounds of formula I may be particularly useful in the treatment of patients with diabetes. For example, the compound may be useful in treating type II diabetes. In type I diabetes, the compounds may be useful as an insulin sensitiser and may therefore allow the dose of insulin administered to be reduced, thereby lowering costs and potentially reducing side effects of insulin
20 administration. Existing anti-diabetic agents, for example the thiazolidinedione class of agents, may have the undesirable effect of stimulating weight gain. Compounds of formula I, such as perfluoroalkyl carboxylic acid compounds and their derivatives (e.g. PFOA or APFO or derivatives thereof), are considered to have the desirable effect of preventing weight gain as well as
25 being useful as anti-diabetic agents.

Compounds of the invention may be useful in the concomitant treatment of a number of abnormalities, for example diabetes, obesity and hyperlipidaemia. Thus, it may be possible to treat a patient with these conditions (which may
30 often occur together) with a single compound or preparation. This may have

benefits, for example in relation to patient compliance, the avoidance of drug interactions, ease of formulation and marketing.

It is preferred that the patient is mammalian, most preferably human or, less preferably a domesticated animal, for example an animal kept as a pet or in agriculture, for example horse, cow, cat or dog.

It is preferred that the compound is a compound wherein the plasma insulin levels are modulated (preferably reduced) in a mammal following administration of the compound to the mammal, relative to either preadministration levels or a control mammal which has not been administered the compound. It is particularly preferred that plasma insulin levels are modulated (for example reduced) (for example relative to a control animal) in a male Fischer 344 rat following administration of the compound to the rat, as described in Example 1. It is sufficient for a reduction to be found at any time following first administration of the compound to the mammal (preferably rat), but it is preferred that such reduction is found (ie appears or is still present) at least seven days after first administration of the compound. It is preferred that a reduction of insulin levels of at least 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80% is achieved.

It will be appreciated that the comparative measurements are made on animals at substantially the same stage of feeding, ie at substantially the same time of day or at substantially the same time following ingestion of food.

When the mammal is a rat, it is preferred that plasma insulin levels are modulated (preferably reduced) following administration of the compound at a level of between 30 and 5000 or 3000ppm of the diet, preferably between about 50 and 500ppm, still more preferably about 300ppm. It is preferred that the test is conducted using the methods and conditions described in Example 1. It is

preferred that the change in insulin level is not accompanied by any adverse clinical symptoms or change in behaviour/activity of the mammal. Thus, the animals may be observed in relation to standard clinical chemistry analyses, blood pressure and/or dizziness. Thus, it is preferred that insulin levels are modulated (preferably reduced) following administration of an amount of the compound that does not produce a significant adverse effect on the animal.

Tests may be performed on more than one animal for a compound or given dose of a compound, as known to those skilled in the art.

10

Alternatively or in addition, it is preferred that plasma cholesterol, glucose and/or triglyceride levels are modulated (preferably reduced), and/or leptin levels modulated, in a mammal following administration of the compound to the mammal, relative to either preadministration levels or a control mammal which has not been administered the compound. Preferences indicated above in relation to modulation (for example reduction) of insulin levels apply similarly in relation to modulation (for example reduction) of plasma cholesterol, glucose and triglyceride levels, and to modulation of leptin levels. Alternatively or in addition, eicosanoid status (ie type or concentration) may be modulated (preferably reduced) in a mammal (or in a particular locus or tissue) following administration of the compound to the mammal, relative to either preadministration levels or a control mammal which has not been administered the compound.

Alternatively or in addition, it is preferred that bodyweight or bodyweight gain is modulated (preferably reduced) in a mammal following administration of the compound to the mammal, relative to either preadministration levels (for bodyweight) or a control mammal (for bodyweight or bodyweight gain) which has not been administered the compound. Preferences indicated above in relation to modulation (for example reduction) of insulin levels apply similarly

in relation to modulation (for example reductions) in bodyweight or bodyweight gain.

Food consumption (expressed as weight of food consumed per unit
5 bodyweight) may be increased following administration of the compound to the mammal, relative to either preadministration levels or a control mammal which has not been administered the compound. The increase may not be seen immediately after commencing administration of the compound; an initial decrease may be seen, which may be followed by an increase.

10

Whilst not wishing to be bound by theory, it is considered that a compound of the invention, for example a perfluorinated fatty acid, for example APFO or PFOA, may bind to a peroxisome proliferator activated receptor (PPAR), for example PPAR α , PPAR δ or PPAR γ (Kliwer *et al* (1994) *PNAS*, 91, 7355-
15 7359; reviewed in Gelman *et al* (1999) *Cell Mol Life Sci* 55, 932-943; Kersten *et al* (2000) *Nature* 405, 421-424 and Issemann & Green (1990) *Nature* 347, 645-650) and may be a PPAR agonist or antagonist. It is preferred that the compound binds to a peroxisome proliferator activated receptor (PPAR), for example PPAR α , PPAR δ or PPAR γ . It is further preferred that the compound
20 is a PPAR α , PPAR δ or PPAR γ modulator, for example a PPAR α , PPAR δ or PPAR γ agonist or antagonist. It is particularly preferred that the compound is a PPAR α or PPAR γ agonist or a PPAR δ antagonist. Any suitable method may be used for determining whether a compound binds to and/or is a modulator, for example an agonist or antagonist of a PPAR.

25

Also whilst not wishing to be bound by theory, a compound of the invention, for example a perfluorinated fatty acid, for example APFO or PFOA, may bind to a lipid metabolising or binding entity, for example a cyclooxygenase, for example COXI or COXII, or phospholipase A, for example Phospholipase A2,

or lipoxygenase. and may be a modulator, for example an activator or inhibitor, of such an entity's activity (including degree of activation). It is preferred that the compound binds to a lipid metabolising enzyme, for example a cyclooxygenase, for example COXI or COXII. It is further preferred that the compound is a modulator of the activity of a lipid metabolising enzyme, for example a cyclooxygenase, for example COXI or COXII. Any suitable method may be used for determining whether a compound binds to and/or is a modulator, for example an activator or inhibitor of a lipid binding or metabolising entity.

10

A further aspect of the invention provides the use of a compound of formula I as defined herein in the manufacture of a medicament for treating a patient in need of modulation of PPAR (for example PPAR α , PPAR δ (also known as β) or PPAR γ) activity. Preferably the patient is in need of an increase in PPAR (preferably PPAR α and/or PPAR γ) activity and the compound of formula I as defined herein is a PPAR (for example a PPAR α or γ) agonist. Alternatively, the patient is in need of a decrease in PPAR (preferably PPAR δ) activity and the compound of formula I as defined herein is a PPAR (for example a PPAR δ) antagonist.

20

A further aspect of the invention provides the use of a compound of formula I as defined herein in the manufacture of a medicament for treating a patient in need of modulation of a lipid metabolising entity activity, for example cyclooxygenase (for example cyclooxygenase I or cyclooxygenase II) activity or phospholipase A (for example phospholipase A2) activity or lipoxygenase activity.

25

A further aspect of the invention provides a screening method for identifying a drug-like compound or lead compound for the development of a drug-like

compound in which (1) a mammal is exposed to a compound of formula I as defined herein (for example a perfluorinated fatty acid) or derivative thereof (2) the plasma insulin, glucose, cholesterol, triglyceride and/or leptin level of the mammal is measured, and/or bodyweight of the mammal is measured, and/or
5 lipid or eicosanoid status (ie type and level of at least one lipid or eicosanoid) or function (for example assessed by degree of responsiveness to the mammal to a lipid or eicosanoid) of the mammal is measured.

The method preferably comprises the step of selecting a compound on exposure
10 to which the plasma insulin, glucose, cholesterol, and/or triglyceride level of the mammal is changed, preferably reduced, and/or leptin level of the mammal is modulated, and/or bodyweight or bodyweight increase is changed, preferably reduced. Preferences for this aspect of the invention include those indicated above in relation to investigating effects on insulin, cholesterol, glucose,
15 triglyceride or leptin levels, or on bodyweight. For example, it is preferred that the mammal is a rodent, for example a rat or a mouse, or other laboratory animal such as a dog.

A further aspect of the invention provides a screening method for identifying a
20 drug-like compound or lead compound for the development of a drug-like compound in which (1) a mammal is exposed to a compound of formula I as defined herein (for example a perfluorinated fatty acid) or derivative thereof (2) the plasma insulin, glucose, cholesterol, triglyceride and/or leptin level of the mammal is measured, and/or bodyweight of the mammal is measured, and/or
25 lipid or eicosanoid status (ie type and level of at least one lipid or eicosanoid) or function (for example assessed by degree of responsiveness to the mammal to a lipid or eicosanoid) of the mammal is measured.

A further aspect of the invention provides a screening method for identifying a
30 drug-like compound or lead compound for the development of a drug-like

compound in which (1) a compound of formula I as defined herein or related compound is exposed to a PPAR polypeptide (2) the binding of the compound to the PPAR polypeptide is measured or the change in the activity of the PPAR polypeptide is measured. Suitable methods by which binding of the compound to the PPAR polypeptide or effect on activity of the PPAR polypeptide may be measured are described, for example, in US 6, 028, 109. The method may comprise the step of selecting a compound that binds to the PPAR polypeptide and/or changes its activity, for example nucleic acid binding activity and/or transcription factor activity. It is preferred that the selected compound increases PPAR α or PPAR γ activity ie acts as a PPAR α or PPAR γ agonist, or decreases PPAR δ activity, ie acts as a PPAR δ antagonist.

A further aspect of the invention provides a screening method for identifying a drug-like compound or lead compound for the development of a drug-like compound in which (1) a compound of formula I as defined herein or related compound is exposed to a lipid metabolising or binding entity, for example cyclooxygenase (for example cyclooxygenase I or cyclooxygenase II) or phospholipase A (for example phospholipase A2) (2) the binding of the compound to the lipid metabolising or binding entity is measured or the change in the activity of the lipid metabolising or binding entity is measured. Suitable methods by which binding of the compound to the lipid metabolising or binding entity or effect on activity of the lipid metabolising or binding entity may be measured will be well known to those skilled in the art. Methods similar to those described in, for example, US 6, 028, 109, may be suitable, as noted above. The method may comprise the step of selecting a compound that binds to the lipid metabolising or binding entity and/or changes its activity, for example production of arachidonic acid from appropriate phospholipid (phospholipase A) or production of prostaglandin from arachidonic acid (cyclooxygenase). It is preferred that the selected compound decreases the

enzymic or binding activity ie acts as an inhibitor of the enzyme or binding entity.

A screening method of the invention may involve comparing the effect
5 achieved using the test compound with that achieved using APFO or PFOA or other compound with desirable properties, as indicated above. A screening method of the invention may involve determining whether the test compound is able to compete with APFO or PFOA or other compound with desirable properties, as indicated above, for example whether it competes with APFO or
10 PFOA for binding to a PPAR polypeptide, for example PPAR α , or other lipid metabolising or binding entity, for example COXI, COXII or phospholipase A2.

Useful screening methods (for example in which the effect of the test
15 compound is compared with that of APFO or PFOA or other compound with desirable properties, as indicated above) also include lipid displacement assays, cell (for example adipocyte) differentiation assays, or other phenotypic assays, insulin sensitisation assays, antinflammatory screen, or investigation of effects on eicosanoid biosynthesis. The compound may be tested in animal models
20 useful in investigating conditions of interest as noted above, such as obesity, diabetes, hyperlipidaemia or carcinogenesis. Such models include obese (ob/ob) or diabetic (db/db) mice, APC/min mice, BB rat or human tumour xenograft models, as known to those skilled in the art.

25 A further aspect of the invention provides a screening method for identifying a drug-like compound or lead compound for the development of a drug-like compound in which (1) a cell is exposed to a compound of formula I as defined herein (for example a perfluorinated fatty acid) or derivative thereof (2) the phenotype (for example differentiation) and/or eicosanoid biosynthesis of the
30 cell is measured. The method preferably comprises the step of selecting a

compound on exposure to which the phenotype, for example differentiation, of the cell is changed, and/or eicosanoid biosynthesis of the cell is changed, preferably reduced.

- 5 The screening methods may be useful in identifying a drug-like compound or lead compound for the development of a drug-like compound for treating diabetes, obesity, hypercholesterolaemia and/or hyperlipidaemia.

10 The methods may further comprise the step of determining whether the compound is toxic or carcinogenic, for example at a concentration sufficient to elicit a change in bodyweight or bodyweight gain, plasma insulin, glucose, cholesterol, triglyceride and/or leptin levels. Such methods will be well known to those skilled in the art.

- 15 It will be appreciated that the compound may preferably be tested in more than of the screening methods of the invention. For example, a compound may be tested for its effect on a PPAR polypeptide, and for its effect on a mammal to which it is administered. The toxicity or carcinogenicity of the compound may also be determined.

20

The term "drug-like compound" is well known to those skilled in the art, and may include the meaning of a compound that has characteristics that may make it suitable for use in medicine, for example as the active ingredient in a medicament. Thus, for example, a drug-like compound may be a molecule that
25 may be synthesised by the techniques of organic chemistry, less preferably by techniques of molecular biology or biochemistry, and is preferably a small molecule, which may be of less than 5000 daltons molecular weight. A drug-like compound may additionally exhibit features of selective interaction with a particular protein or proteins and be bioavailable and/or able to penetrate

cellular membranes, but it will be appreciated that these features are not essential.

The term "lead compound" is similarly well known to those skilled in the art, and may include the meaning that the compound, whilst not itself suitable for use as a drug (for example because it is only weakly potent against its intended target, non-selective in its action, unstable, difficult to synthesise or has poor bioavailability) may provide a starting-point for the design of other compounds that may have more desirable characteristics.

10

These "lead" compounds may then be developed further, for example by molecular modelling/and or experiments to determine a structure activity relationship, in order to develop more efficacious compounds, for example by improving potency, selectivity/specificity and pharmacokinetic properties.

15

The methods may be performed *in vitro*, either in intact cells or tissues (for example liver cells or adipocytes), with broken cell or tissue preparations or at least partially purified components. Alternatively, they may be performed *in vivo*. The cells tissues or organisms in/on which the use or methods are performed may be transgenic. In particular they may be transgenic for a PPAR polypeptide or lipid metabolising or binding entity.

20

It will be appreciated that the polynucleotide encoding the PPAR (for example PPAR α , β or γ) or lipid metabolising or binding entity may be mutated in order to encode a variant of the PPAR, for example by insertion, deletion, substitution, truncation or fusion, as known to those skilled in the art. It is preferred that the PPAR or lipid metabolising or binding entity is not mutated in a way that may materially affect its biological behaviour, for example its nucleic acid binding or transcription factor activity or lipid metabolising or binding activity, as appropriate.

30

The following references relate to the sequences and tissue distribution of PPARs: Auboeuf *et al* (1997) *Diabetes* **46**(8), 1319-1327; Braissant *et al* (1996) *Endocrinol* **137**(1), 354-366; Mukherjee *et al* (1994) *J Steroid Biochem*
5 *Mol Biol* **51**, 157-166; Mukerjee *et al* (1997) *J Biol Chem* **272**, 8071-8076.

The following references and GenBank Accession numbers relate to the sequences and/or tissue distribution of the indicated polypeptides.

- 10 U63846 Human COX-1 cDNA (PTSG1); Hla (1996) *Prostaglandins* **51**, 81-85.
NM000963 Human COX2 cDNA (PTSG2); Hla & Neilson (1992) *PNAS* **89**(16), 7384-7388; Jones *et al* (1993) *J Biol Chem* **268**(12), 9049-9054; Appleby *et al* (1994) *Biochem J* **302**, 723-727; Kosaka *et al* (1994) *Eur J Biochem* **221**(3), 889-897.
- 15 AF306566 Human phospholipase A2 (secreted form); Valentin *et al* (2000) *Biochem Biophys Res Commun* **279**(1), 223-228.
NM021628 Human lipogenase ALOXE3.
XM005818 Human lipxygenase ALOXE5.
XM008328 Human lipoxegenase ALOX12.
- 20 NM001141 Human lipoxegenase ALOX15; Brash *et al* (1997) *PNAS* **94**(12), 6148-6152.
NM005090 and NM 003706 Human phospholipase A2 (cPLA2-gamma) Underwood *et al* (1998) *J Biol Chem* **273**(34), 21926-21932 and Pickard *et al* (1999) *J Biol Chem* **274**(13), 8823-8831
- 25 M68874 Human phospholipase A2 (cPLA2) Sharp *et al* (1991) *J Biol Chem* **266**(23), 14850-14853.

It will be appreciated that such a compound may be an agonist or antagonist of the PPAR polypeptide used in the screen and that the intention of the screen is
30 to identify compounds that act as agonists or antagonists of the PPAR, even if

the screen makes use of a binding assay rather than an activity assay, for example transcription factor activity or nucleic acid (for example DNA) binding activity. It will be appreciated that the action of a compound found to bind the PPAR polypeptide may be confirmed by performing an assay of transcription factor activity or nucleic acid binding activity in the presence of the compound.

Likewise, such a compound may be an inhibitor or activator of the lipid metabolising or binding entity used in the screen and that the intention of the screen is to identify compounds that act as inhibitors or activators of the lipid metabolising or binding entity, even if the screen makes use of a binding assay rather than an activity assay, for example lipid metabolising activity, for example prostaglandin production from arachidonic acid for COXI or COXII. It will be appreciated that the action of a compound found to bind the lipid metabolising or binding entity may be confirmed by performing an assay of the appropriate enzyme or binding activity in the presence of the compound.

It is preferred that the assay is capable of being performed in a "high throughput" format. This may require substantial automation of the assay and minimisation of the quantity of a particular reagent or reagents required for each individual assay. A scintillation proximity assay (SPA) based system, as known to those skilled in the art, may be beneficial. Combinatorial chemistry techniques may be used in generating compounds to be tested.

A further aspect of the invention provides a kit of parts of screening system comprising (1) a library of compounds each of formula I as herein defined or a derivative thereof, and (2) a PPAR polypeptide or polynucleotide encoding a PPAR polypeptide, and/or a test mammal. The kit may optionally comprise reagents useful in measuring plasma insulin, glucose, triglyceride and/or cholesterol levels, or in measuring PPAR activity, for example nucleic acid

binding. Such reagents will be apparent to those skilled in the art, and may include reagents useful in performing transactivation assays or DNA binding assays.

- 5 A further aspect of the invention provides a kit of parts of screening system comprising (1) a library of compounds each of formula I as herein defined or a derivative thereof, and (2) a lipid metabolising or binding entity (for example COXI or COXII or phospholipase A2 or lipoxygenase) or polynucleotide encoding a lipid metabolising or binding entity. The kit may optionally
- 10 comprise reagents useful in measuring plasma insulin, glucose, triglyceride, cholesterol and/or leptin levels, or in measuring the activity of the lipid metabolising or binding entity, for example a substrate of the lipid metabolising or binding entity (for example arachidonic acid in the case of COXII or lipoxygenase) or reagent useful in measuring a product of a lipid metabolising
- 15 enzyme, for example in assessing eicosanoid biosynthesis. As well known to those skilled in the art, reagents may include labelled ligand, for example radiolabelled or fluorescently labelled. Direct binding or displacement of ligand may be measured. Binding may be measured using fluorescence resonance energy transfer (FRET) techniques. The kit may optionally include
- 20 reagents useful in cell differentiation assays, for example adipocyte differentiation assays, as will be known to those skilled in the art.

- A further aspect of the invention provides a compound identifiable or identified by a screening method of the invention. A further aspect of the invention
- 25 provides a compound identified or identifiable by a screening method of the invention for use in medicine. A further aspect of the invention provides a pharmaceutical composition comprising a compound identified or identifiable by a screening method of the invention and a pharmaceutically acceptable excipient. Preferences in relation to properties of such compounds are as
- 30 indicated above and in relation to the first aspect of the invention.

A compound identified or identifiable by a screening method of the invention is also provided for use in the manufacture of a composition for use as a food supplement or a food additive. The invention also relates to a food product
5 comprising a foodstuff and a compound of formula I as defined herein or a compound identified or identifiable by a screening method of the invention, wherein the food is not laboratory rodent, for example rat or mouse, feed. It is preferred that the food is not laboratory animal feed.

10 Preferably, the food (the term including food product and foodstuff) is suitable for administration to an animal (for example a domesticated animal as discussed above but not a laboratory rodent) or human, for example an adult human, baby or infant.

15 A further aspect of the invention provides the use of a compound identified or identifiable by a screening method of the invention in the manufacture of a medicament for the treatment of a patient in need of modulation (for example reduction) of body mass or modulation (preferably reduction or prevention) of increase in body mass, and/or in need of modulation (preferably reduction) of
20 plasma insulin, plasma glucose, plasma triglycerides and/or plasma cholesterol, and/or in need of modulation of plasma leptin. The patient may be obese and/or have diabetes, hyperlipidaemia and/or atherosclerosis, or be at risk of developing such a condition.

25 A further aspect of the invention provides the use of a compound identified or identifiable by a screening method of the invention in the manufacture of a medicament for the treatment of a patient who is overweight or obese and/or has diabetes, hyperlipidaemia, atherosclerosis, coronary heart disease, stroke, obstructive sleep apnoea, arthritis (for example osteoarthritis) and/or reduced
30 fertility, or is at risk of developing such a condition.

A further aspect of the invention provides the use of compound identified or identifiable by a screening method of the invention in the manufacture of a medicament for treating a patient in need of modulation of PPAR (for example
5 PPAR α) or modulation of lipid or eicosanoid status or function, or of lipid metabolising or binding entity (for example COXI, COXII, phospholipase A or lipoxygenase) activity. Preferably the patient is in need of an increase in PPAR (preferably PPAR α or PPAR γ) activity and the compound is a PPAR (for example a PPAR α or PPAR γ) agonist. Alternatively, the patient may be in
10 need of a decrease in activity of a lipid metabolising or binding entity and the compound is an inhibitor of that lipid metabolising or binding entity (or entities).

The compounds may be administered in any suitable way, usually parenterally,
15 for example intravenously, intraperitoneally or intravesically, in standard sterile, non-pyrogenic formulations of diluents and carriers. The compounds may also be administered topically. The compounds of the invention may also be administered in a localised manner, for example by injection. Preferably, the compounds are administered orally. The compounds may be administered
20 as a tablet or capsule or as a supplement added to food or drink. A slow-release formulation may be used.

A further aspect of the invention provides a method of treatment of a patient in need of modulation (preferably reduction) of body mass or modulation (for
25 example reduction or prevention) of increase in body mass, and/or in need of modulation (for example reduction) of plasma insulin, plasma glucose, plasma triglycerides, plasma cholesterol and/or leptin, comprising administering to the patient an effective amount of a compound identified or identifiable by the screening method of the invention. A further aspect of the invention provides a

method of treatment of a patient who is overweight or obese and/or has diabetes, hyperlipidaemia, atherosclerosis, coronary heart disease, stroke, obstructive sleep apnoea, arthritis (for example osteoarthritis) and/or reduced fertility, or is at risk of developing such a condition, comprising administering
5 to the patient an effective amount of a compound identified or identifiable by the screening method of the invention.

Preferences in relation to the patient and compound are as indicated above.

- 10 A further aspect of the invention provides a method of treatment of a patient in need of modulation of PPAR (for example PPAR α , δ or γ) activity, or of lipid or eicosanoid status or function, or of a lipid metabolising or binding entity activity, comprising administering to the patient an effective amount of a compound identified or identifiable by the screening method of the invention.
- 15 Preferably the patient is in need of an increase in PPAR (preferably PPAR α or γ) activity and the compound is a PPAR (for example a PPAR α or γ) agonist. Further preferences in relation to the patient and compound are as indicated above. Alternatively, the patient may be in need of a decrease in activity of a lipid metabolising or binding entity and the compound is an inhibitor of that
20 lipid metabolising or binding entity or entities.

The invention is now described in more detail by reference to the following, non-limiting, Figures and Examples:

25 **Figure 1:** Growth curves for male Fischer 344 rats administered APFO in diet.

Figure 2: Food consumption by Fischer 344 rats administered APFO in diet.

Figure 3: Food utilisation in Fischer 344 rats administered APFO in diet.

Figure 4: Effect of APFO treatment on plasma insulin concentration.

Figure 5: Effect of APFO on plasma cholesterol concentration.

5

Figure 6: Effect of APFO on plasma glucose concentration.

Figure 7: Effect of APFO on plasma triglyceride concentration.

10 **Figure 8:** compounds indicated to be PPAR agonists in US 6,028,109

Figure 9: Effect of APFO treatment on mouse body weights

Figure 10: Effect of APFO treatment on mouse food consumption

15

Figure 11: Effect of APFO treatment on mouse food consumption (expressed as grams food consumed per unit body weight).

Figure 12: Effect of APFO treatment on mouse plasma insulin concentration.

20 Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 13: Effect of APFO treatment on mouse plasma triglyceride concentration. Values are Mean \pm SD. Significantly different from respective

25 control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 14: Effect of APFO on mouse plasma glucose concentration. Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

- 5 **Figure 15:** Effect of APFO treatment on mouse plasma cholesterol. Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

- 10 **Figure 16:** Effect of APFO treatment on mouse plasma leptin. Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

- 15 **Figure 17:** Effect of APFO treatment of mouse epididimal fat pad weight. Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 18: Effect of APFO treatment on rat body weight.

Figure 19: Effect of APFO treatment on rat food consumption.

20

Figure 20: Effect of APFO on rat food consumption (expressed as grams food consumed per unit body weight).

- 25 **Figure 21:** Effect of APFO treatment on plasma insulin concentration. Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 22: Effect of APFO treatment on rat plasma glucose concentration. Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

- 5 **Figure 23:** Effect of APFO treatment on rat plasma triglyceride concentration. Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

- 10 **Figure 24:** Effect of APFO treatment on rat plasma cholesterol. Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 25: Effect of APFO treatment on rat plasma leptin concentration.

- 15 **Figure 26:** Cytotoxic effect of APFO on HepG2 cells (A), HT-29 cells (B) and MCF7 cells (C).

- 20 **Figure 27:** Effect of prophylactic APFO treatment on tumour volume in an HT-29 xenograft model. * = APFO administration. Arrow indicates the point of tumour cell implantation. Tumour measurement began on day 1.

- 25 **Figure 28:** Effect of prophylactic APFO administration on tumour volume between day 1 and day 15 of treatment. Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 29: Effect of prophylactic APFO administration on tumour weight. Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 30: Effect of therapeutic APFO treatment on tumour volume in an HT-29 xenograft model. * = APFO administration. Arrow indicates the point of tumour cell implantation. Tumour measurement and APFO administration began on day 1.

Figure 31: Effect of therapeutic APFO administration on tumour volume between day 1 and day 17 of treatment. Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 32: Effect of therapeutic APFO administration on tumour weight. Values are Mean \pm SD. Significantly different from respective control group (0 mg/kg); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 33: Effect of prophylactic APFO administration on nu/nu mouse body weight.

Figure 34: Effect of therapeutic APFO administration on nu/nu mouse body weight.

Figure 35: Activation of Mouse PPAR α by APFO.

Figure 36: Interaction of APFO with Ligand Binding Domain of Human PPAR γ .

Example 1: Effect of perfluorinated fatty acid on insulin, glucose, cholesterol and triglyceride levels, and on body weight

METHODS

Male Fisher 344 rats (initially 6 weeks old) were administered ammonium perfluorooctanoate (APFO, 300ppm) in the diet for periods of time up to one
5 year. Control rats received powdered diet that did not contain APFO.

Body weights were initially determined daily and then weekly. Food consumption was determined weekly. Clinical observations were made daily.

10 Rats were sacrificed at 1, 2, 7, 14, 28, 90, 182 and 365 days. There were 8 rats per group. At sacrifice blood was sampled by cardiac puncture and submitted for clinical chemistry.

The following assay methods/kits were used:

Assay	Supplier	Kit Number
ALT	Roche Diagnostics	MPR 1087 568
AST	Roche Diagnostics	Unimate 3 0736414
Glucose	Roche Diagnostics	MPR2 1442 449
Triglycerides	Roche Diagnostics	Peridochrom GPO-PAP 701 882
Cholesterol	Roche Diagnostics	CHOD-AP MPR3 236691
Insulin	Amersham	RNP 2567

15

RESULTS

Administration of APFO to male Fischer 344 rats lead to marked reductions in
20 bodyweight gain (Figure 1). Treated animals had body weights approximately 25-30% lower than concurrent controls. This weight change was not accompanied by any adverse clinical symptoms or changes in activity.

Food consumption expressed per rat was markedly decreased (to approximately 50% of control consumption) during the first week of treatment. However after this time food consumption per rat increased to 80-90% of control values (Figure 2).

5

When expressed as weight of food consumed per unit bodyweight, food consumption was decreased by approximately 30% during the first week of APFO administration. However, at later times APFO-treated animals consumed between 10 and 30% more food per unit bodyweight than controls (Figure 3).

10

Plasma cholesterol, glucose and insulin concentrations were decreased at all time points examined (Figures 4-6), while plasma triglycerides were decreased at 7 days and beyond (Figure 7).

15

These data suggest that APFO and related compounds may be useful for treatment of obesity, diabetes, hypertriglyceridaemia and hypercholesterolaemia and diseases where alterations in lipid or eicosanoid status may be desirable, such as arthritis or cancer.

20

Example 2: Effect of APFO in reversing obesity and diabetes

The effect of APFO in animal models of obesity and diabetes was studied in order to establish the therapeutic potential of APFO to reverse obesity and diabetes.

25

The studies reported in Example 1 involving the administration of APFO to Sprague Dawley rats for up to one year, demonstrated the compound's anti-diabetic and anti-obesity potential. Following an initial reduction in food consumption during the first week of the study, an increase in food

30

consumption per unit body weight was coupled to a marked reduction in body weight gain in treated animals throughout the test period. Furthermore, plasma cholesterol and insulin concentrations were decreased at all time points examined, while plasma glucose and triglycerides were decreased at 7 days and
5 beyond.

In this Example, these observations in healthy rats are extended by investigations in two models of metabolic disease - the obese mouse (ob/ob) and the diabetic GK/Mol rat.

10

1.1 Mouse Model (ob/ob)

The C57BL/6J-ob/ob mouse is an obese, leptin-deficient animal that is widely accepted as a model of obesity and diabetes. Age-paired, disease-free (lean)
15 animals (C57BL/6J-+/+) were also included in the study to observe the 'normal' response.

1.1.1 Experimental Design and Methods.

20 Three groups (n=5) of ob/ob (C57BL/6J-ob/ob) mice were treated with 3 dose levels of APFO (5, 15 and 25mg/kg/day). Animals were administered APFO, dissolved in water, by oral gavage, daily for 14 days. One group of 10 ob/ob mice was also treated with vehicle (water) alone. Additionally, to observe the 'normal' response, 5 age-paired, disease-free animals (C57BL/6J-+/+) were
25 administered 25mg/kg APFO and a similar disease-free control group was administered vehicle only.

Twenty-four hours after the last dose the animals were killed by an increasing concentration of carbon dioxide. Blood was collected by cardiac puncture and

plasma prepared and stored at -70°C until analysed. Major tissues were weighed, sampled, flash frozen in liquid nitrogen and stored at -70°C .

Plasma was analysed for triglycerides, cholesterol and glucose using kits
5 purchased from Sigma (Poole, Dorset). Concentrations of plasma insulin and leptin were determined using commercially available enzymeimmunoassay-based kits from Amersham Life Sciences and Crystalchem Inc., (Chicago) respectively. All assays were carried out as specified by the manufacturer.

10 *1.1.2 Results and Discussion.*

Both strains of mice treated at 25 mg/kg/day lost bodyweight over the treatment period. In +/+ mice this was only apparent after day 4; these animals also lost less weight, as a percentage of initial bodyweight, than the ob/ob mice
15 (26% versus 33%). In ob/ob mice treated at 15 mg/kg/day 20% bodyweight loss was noted over the study period. Animals treated at 5 mg/kg/day were unaffected (Figure 9). No other adverse clinical observations were observed.

The bodyweight losses were reflected in marked, APFO dose-related, decreases
20 in food consumption (76%, 53% and 17% lower than control mice at the high, intermediate and low dose levels respectively) (Figure 10). In +/+ mice a decrease in food consumption was evident over the first nine days of treatment, following which there was a steep recovery towards control values without equalling them. The overall consumption was still 31% lower than in +/+
25 controls. When expressed in terms of food consumed per gram of bodyweight the pattern of effect was similar, although the recovery in values seen in +/+ mice after day 9 was greater and the subsequent values more nearly equal to those of their controls (Figure 11).

There was a very marked reduction (greater than 90%) in plasma insulin concentrations in all treated ob/ob mice, which was broadly related to dose level (Figure 12). In +/+ mice, control insulin levels were notably lower than in the ob/ob mice. However, APFO-treatment still led to a marked reduction in plasma insulin concentrations.

APFO-treated (15 or 25 mg/kg/day) ob/ob mice showed dose-related reductions in plasma glucose down to approximately 20% of control values, similarly plasma triglyceride concentrations were decreased to 40% of control values (Figure 13 and 14). At 5 mg/kg/day administered to ob/ob mice, glucose was reduced by approximately 50% but there was no effect on triglyceride concentrations (Figure 14). In +/+ mice, APFO (25mg/kg/day) decreased glucose and triglycerides to 55% and 35% of control plasma values respectively.

At a dose of APFO of 25 mg/kg/day to both strains there was an approximate 30% reduction in plasma cholesterol concentrations. This was not evident at the low and mid dose levels in the ob/ob mice (Figure 15).

Plasma leptin concentrations in the ob/ob mice were below the levels of quantitation; this was expected as there is an early stop codon within the leptin gene of this mouse strain. Treatment of the +/+ mouse, which possesses a normal leptin gene, with APFO (25mg/kg/day) resulted in decreased plasma leptin concentrations to, or below the level of quantitation (Figure 16).

Epididymal fat pad (white adipose tissue) weights were 7-fold higher in control ob/ob mice compared to control +/+ mice. APFO-treatment decreased the weight of the epididymal fat pads in a dose-related manner in ob/ob mice and +/+ mice (Figure 17).

1.1.3 Conclusions.

In summary, there were a number of significant physiological effects that could be related to the administration of APFO. In lean controls, there was a slight
5 reduction in body weight, and this loss reached a nadir after 10 days with no weight loss occurring after this time. Food consumption in this group remained constant.

At the high and intermediate dose levels, ob/ob mice continued to lose body
10 weight. At 25mg/kg, ob/ob mice also displayed appetite loss (reflected in body weight changes). In this group there was also marked reduction in glucose levels. This appeared to suggest that the anti-obesity effects may have been due to reduced food consumption. However, in animals treated with 5mg/kg APFO, a 17% reduction in food consumption was associated with a 50% reduction in
15 plasma glucose levels, which suggested that the anti-obesity effects observed in ob/ob mice were due to metabolic changes caused by APFO, and not to a loss of appetite.

These data suggest that APFO causes weight loss in obese animals, but not,
20 significantly, in lean animals and so may be used as an anti-obesity agent. Additionally the APFO-induced decreases in plasma glucose and insulin suggest that this chemical may be of therapeutic use in Type II diabetes.

1.2 Rat GK/Mol Model.

25

The GK/Mol rat is a non-obese, diabetic animal that is widely accepted as a model of Type II diabetes. In order to measure the 'normal' response, non-diabetic Wistar rats were also used in the study.

30 1.2.1 Experimental Design and Methods.

Three groups (n=5) of GK/Mol rats were administered 3 dose levels of APFO (3, 10 and 30mg/kg). Animals were administered APFO by oral gavage, daily for 14 days. One group of 10 GK/Mol rats was also treated with vehicle (water) alone. Additionally, to observe the 'normal' response, 5 age-paired, disease-free Wistar rats were administered 30mg/kg APFO and a similar disease-free group was administered vehicle only.

Twenty-four hours after the last dose the animals were killed by an increasing concentration of carbon dioxide. Blood was collected by cardiac puncture and plasma prepared and stored at -70°C until analysed. Major tissues were weighed, sampled, flash frozen in liquid nitrogen and stored at -70°C .

Plasma was analysed for, triglycerides, cholesterol, glucose, insulin and leptin as described in section 1.1.1.

1.2.2 Results and Discussion.

APFO administration to GK/Mol rats resulted in a dose-dependent decrease in body weight gain to 90%, 71% and 44% of control values at the low, mid and high dose levels respectively (Figure 18). There was no effect on bodyweight gain in treated Wistar rats.

Treated GK/Mol rats had slightly lower total food consumption (86-98% of control values), although this difference was not related to dose level (Figure 19). There was no difference in food consumption between treated Wistar rats and their controls. No pattern was discernible when the data were expressed as food eaten per gram of bodyweight (Figure 20).

There was a marked dose-dependent reduction in the plasma concentration of insulin, reaching about 10% of control values in both strains of rat (Figure 21).

Plasma glucose concentrations in GK/Mol rats were lowered by APFO to about
5 85% of control values at dose levels of 30mg/kg/day (Figure 22). Plasma Triglycerides (Figure 23) and cholesterol (Figure 24) concentrations were lower by between 10 and 20% in treated GK/Mol rats. In Wistar rats, plasma cholesterol was reduced to 73% of control values.

10 Group mean plasma concentrations of leptin (Figure 25) were slightly lower (by approximately 40%) in Wistar rats treated at 30 mg/kg/day than in their controls. There were no differences in leptin concentrations in the GK/Mol rats that could be indicative of a treatment-related effect.

15 1.2.3 Conclusions.

The GK/mol study followed a similar pattern to the investigation in Sprague Dawley rats (Example 1). APFO caused a reduction in the levels of glucose, triglycerides and cholesterol coupled to reduced weight gain in treated animals;
20 there was also a marked reduction in the level of plasma insulin.

In conclusion APFO demonstrated anti-diabetic effects in a rat model for type II diabetes, further indicating it may be an effective agent for the treatment of this condition.

25

2. Therapeutic Potential of APFO as an Anti-cancer Agent.

The effect of APFO *in vitro* against human tumour cell lines and *in vivo* in a human tumour xenograft model was examined.

30

2.1 *In vitro* Anti-tumour Activity.

Three human cancer cell lines were exposed to APFO and cytotoxicity levels assessed in order to assess APFO's functions as an anti-cancer agent.

5

2.1.1 *Experimental Design and Methods.*

HT-29 cells (human colon tumour-derived), MCF7 cells (human breast cancer-derived) and HepG2 cells (human liver cancer-derived) were cultured in
10 Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% heat-inactivated foetal calf serum, 2mM L-glutamine, penicillin (50IU/ml), streptomycin (50 µg/ml) and .1% non-essential amino acids. Cells were harvested by trypsinisation and diluted to 5×10^4 cells/ml, and 200µl of cell suspension was plated into each well of a 96 well plate and allowed to attach
15 overnight at 37°C with 5%CO₂. Cells were exposed to various concentrations of APFO in growth medium (0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30 and 100 and 1000µM) for four hours. 20µl of a 5mg/lm MTT solution was added to each well and the cells were incubated for 4 hours at 37°C. The medium was removed and 200 µl DMSO was added to dissolve formazan crystals. Plates
20 were read at 570nm and background at 690nm was subtracted. Results were displayed graphically as percentage cell survival versus APFO concentration.

2.1.2 *Results and Discussion.*

25 APFO elicited a cytotoxic effect after 4 hours at concentrations exceeding 500µM (Figure 26).

2.1.3 *Conclusions*

This study indicated that APFO was effective at killing a range of human
30 cancers *in vitro*.

2.2 *HT-29 Xenograft Model in Nude Mice.*

The objective of this study was to examine the anti-tumour capabilities of APFO in a xenografted animal model. Effects of APFO on tumour progression were tested on a human colon cancer-derived cell line xenografted into immune-deficient nu/nu mice. Prophylactic and therapeutic effects of APFO were assessed by measurement of tumour size at regular intervals during administration of APFO.

10

2.2.1 Experimental Design and Methods.

Athymic nude (nu/nu) mice from ICRF stock (HsdOla:ICRF-nu) were obtained from Clare Hall (Potters Bar, UK). All animals were female and approximately 9 weeks old. Animals, housed in isolator cages and handled under laminar flow, were divided into one control group and two treatment groups, with 5 female mice per group for both the prophylactic and therapeutic schedules

HT-29 cells were cultured according to the conditions described in section 2.1.1. Cells were harvested, pooled by centrifugation and resuspended in 5ml medium to which 5ml Matrigel (basement membrane matrix) was added. 100µl of cell suspension was injected subcutaneously into each flank of the mice (1.75×10^6 cells per flank). In order to assess the prophylactic effect of APFO, half of the animals were administered the compound immediately following tumour cell implantation. For the therapeutic schedule, APFO was injected once tumours had developed.

Animals were administered APFO, dissolved in water, by intra-peritoneal (ip) injection 3 times per week for one month. The doses of APFO were 15mg/kg

30

and 25mg/kg bodyweight. The volume of the dosing solution was 10ml/kg bodyweight. Control animals received an equivalent volume of water.

Animal bodyweights were recorded throughout the study. Tumour growth was measured 3 times per week using digital calipers and the volume was calculated using the formula:

$$4/3\pi \cdot \left\{ \frac{(d_1 + d_2)^3}{4} \right\}$$

Where d_1 = mean length (n=2) and d_2 = mean width (n=2). (NB, n=4 if tumour was an irregular shape).

The maximum permitted tumour volume, according to the terms of the Home Office licence, was 1.44cm³. Results were expressed graphically for each time point as mean tumour volumes. Tumour weights were recorded at the end of the study, and tumour samples were either snap frozen in liquid nitrogen or fixed in formal saline for further analysis.

2.2.2 Results and Discussion.

HT-29-derived tumours developed approximately 14 days into the study.

Tumour growth in both prophylactic and therapeutic groups proceeded at a much faster rate in control groups compared to tumours in APFO-treated animals (Figures 27 and 30 respectively). Consequently, the therapeutic study

was not completed because control animals were lost either because tumour volume exceeded the permitted size, or because the tumours were deemed ulcerated and again continuance was not permitted under the terms of the Project Licence. Hence, animals in the therapeutic study were injected at 8 time points compared to 13 time points in the prophylactic study.

Tumour growth rate in animals treated prophylactically was markedly slower in APFO-treated animals, with a lag phase of 15 days for control groups compared to 22 days for APFO-treated mice (Figure 27). In animals administered 25mg/kg APFO, tumour growth reached a plateau after 26 days, while in animals dosed at 15mg/kg, tumour volume continued to increase (Figure 27). Tumour volume in the prophylactic groups increased 18 fold in controls, 8 fold at 15mg/kg and 6 fold at 25mg/kg between the start of tumour measurement (day 1) and the end of the study (day 15) (Figure 28). Upon necroscopy, in the 15mg/kg and 25mg/kg groups respectively, tumour weights were 22% and 58% smaller than in control animals (Figure 29).

Tumour growth rate was also markedly slower in animals treated therapeutically with APFO, with a lag phase of 17 days in control tumours compared to 26 days in treated mice (Figure 30). No plateau was reached in the highest dose group, but the study was incomplete as animals were treated for a shorter period than intended. Tumour volume increased 15-fold in controls, 14-fold at 15mg/kg, and 7-fold at 25mg/kg between day 1 and day 17 (Figure 31). Tumour weights were 45% and 37% smaller in the 15mg/kg and 25mg/kg dose groups respectively (Figure 32). It should be noted that 5 animals (4 controls and 1 high dose animal) had been lost from the study, thus affecting mean values of final tumour weights.

Tumours were removed from animals at the end of the study period and examined macroscopically. Control tumours from the prophylactic group were

solid, while APFO-treated animals produced tumours that were fluid-filled, suggesting cell death in the centre of the tumours. Differences between control and treated groups were less obvious in animals treated therapeutically, but these animals were dosed for a shorter period. Samples of tumours were
5 formalin fixed and also flash frozen in liquid nitrogen for histopathological examination.

Animal body weights were monitored and recorded throughout the study. There was no significant difference between control and treated animals in either the
10 prophylactic or therapeutic groups in animals implanted with HT-29 cells (Figures 33 and 34).

2.2.3 Conclusions.

In summary, APFO demonstrated anti-tumour capabilities in a human cancer
15 cell line when either given concomitantly with the tumour cells or following tumour establishment. Additionally, body weight remained unaffected by the test agent, suggesting that treatment-associated weight loss would not occur, a major advantage for chemotherapeutic agent. It is probable that treatment-associated weight loss did not occur because APFO selectively targets obese
20 subjects and not lean subjects (eg. nu/nu mice).

Finally, APFO showed anti-tumour capabilities against HT-29 cells, a human colon cancer cell line, thus demonstrating that it is capable of inhibiting the
25 growth of human tumour cells.

3. The Potential Anti-inflammatory Properties of APFO.

3.1 In vitro Studies

The ability of APFO (or other test compound) to inhibit cyclooxygenase 1 (COX1) and cyclooxygenase 2 (COX-2) inhibition is examined using an EIA-based human COX inhibitor assay kit as described by the manufacturer (Cayman Chemical, Michigan).

5

3.2 *In vivo Studies.*

The anti-inflammatory potential of APFO (or other test compound) is examined in a rat model. Animals are dosed with APFO or dexamethasone, after which
10 the animal's immune system is challenged with lipopolysaccharide (LPS) and plasma cytokines are measured. The study may consist of one control group and three treatment groups, with 10 male CD rats (80 – 120g) per group. The control group is administered vehicle (water) only followed by LPS (30µg per 100g rat) 24hrs later. Treatment group 1 animals receive APFO (or other test
15 compound) at 30mg/kg. Treatment group 2 animals receive APFO (or other test compound) at 30mg/kg followed by LPS (30µg per 100g rat) 24hrs later. Treatment group 3 animals received dexamethasone (10mg/ml in corn oil) followed by LPS (30µg per 100g rat) 1 hour later. The plasma from 5 animals per group is harvested 1 hour or 2 hours post-treatment. Plasma cytokines (IL-6,
20 IL-1β and TNF) are measured using commercially available kits as specified by the manufacturer (Endogen Inc., Massachusetts).

4. *Interaction of APFO with PPAR Isoforms*

25 Transactivation assays involving mouse PPAR alpha cDNA and ligand binding assays using human PPAR gamma were performed in order to demonstrate that APFO interacts with PPAR isoforms.

4.1 Mouse PPAR Transactivation Assay

4.1.1 Experimental design and methods

COS-1 cells (cultured in medium described in section 2.1.1 but without non-essential amino acids) were plated into 6 well tissue culture dishes at 3×10^5 cells per well and allowed to adhere overnight at 37°C. The next day the medium was aspirated and the cells washed with PBS, pH7.4, and 200µl of a transient transfection cocktail was added to each well. The transfection cocktail was composed of 50ng of vector DNA carrying mouse PPAR alpha, 500ng of plasmid DNA containing the PPAR response element of liver fatty acid binding protein and, as a transfection control, 500ng of a vector harbouring β-Galactosidase. DNA was dissolved in PBS containing 50µg/ml DEAE-Dextran. Control cells were exposed to a transfection cocktail that contained no plasmid DNA. Cells were incubated at 37°C for 30 minutes before 2ml of medium containing 80µM chloroquine was added and the cells incubated for a further 2.5 hours at 37°C. The medium was aspirated and the cells shocked with 10% DMSO in medium for 2.5 minutes at room temperature. Cells were washed with PBS then allowed to recover at 37°C in growth medium for 24 hours.

Transiently transfected cells were exposed to APFO (dissolved in water) in medium at 0, 3, 10, 30, 100, 300 and 1000µM for 16 hours at 37°C. Cells were then washed, lysed, and luciferase and β-Galactosidase activities were measured using kits according to the methods specified by the manufacturer (Promega, Madison, USA) by flash luminescence and spectrophotometry respectively. Luciferase expression was normalised by dividing by the flash luminescence reading with constitutive β-Galactosidase expression levels measured at 415nm following a colourimetric assay.

Data were graphed, fitted to non-linear regression curves and EC₅₀ values calculated using GraphPad Prism software.

4.1.2 Results and discussion

5

Activation of mouse PPAR alpha by APFO occurred, with an effective concentration (EC₅₀) of 102µM. (Figure 27).

4.1.3 Conclusions

10

The data presented here demonstrate that APFO is a mouse PPAR alpha activator at µM concentrations.

4.2 PPAR Gamma Ligand Binding Studies

15

His-tagged human PPARγ ligand binding domain was expressed in *E. Coli* as described previously [Palmer, CAN and Wolf, CR. FEBS Letts. 431, 476-480, (1998)]. The receptor protein was partially purified by nickel affinity chromatography.

20

4.2.1 Ligand Binding studies.

This recombinant receptor protein has been used previously to study interactions with the fluorescent fatty acid - *cis*-parinaric acid (CPA)[Palmer
25 CAN and Wolf CR. FEBS Letts. 431, 476-480, (1998); Causevic M, Wolf CR and Palmer CAN. FEBS Letts. 463, 205-210, (1999)]. On binding to the receptor, changes in the spectral properties of the fatty acid occurs. These are

quantitatively related to the binding of the ligand to the receptor and can be used to calculate binding constants. A competitive displacement assay can be utilised to examine the binding characteristics of other compounds. APFO was assayed for its ability to displace *cis*-parinaric acid from PPAR γ by this
5 method. Data were analysed as described in section 4.1.

4.2.2 Results and discussion.

Competitive ligand binding assays using the ligand binding domain of human
10 PPAR gamma showed that displacement of *cis*-parinaric acid occurred, with an EC₅₀ of 355 μ M (Figure 28).

3.2.4 Conclusions

These data indicate that APFO interacts with the ligand binding domain of
15 human PPAR gamma.

CLAIMS

1. A method of treatment of a patient in need of modulation of body mass or modulation of increase in body mass, and/or in need of modulation of plasma insulin, plasma glucose, plasma triglycerides, plasma cholesterol and/or leptin, comprising administering to the patient an effective amount of a compound of formula I ,



wherein

- 10 Z^1 represents $-\text{CO}_2\text{H}$ or a derivative thereof;
 Z^2 represents F, H, $-\text{CO}_2\text{H}$ or a derivative thereof; and
X represents fluorinated alkylene;
or a solvate thereof.
- 15 2. A method of treatment of a patient in need of an antitumour agent or an antiinflammatory agent, or in need of modulation in lipid or eicosanoid status, comprising administering to the patient an effective amount of a compound of formula I as defined in claim 1.
- 20 3. A method of treatment of a patient who is overweight or obese and/or has diabetes, hyperlipidaemia, atherosclerosis, coronary heart disease, stroke, obstructive sleep apnoea, arthritis and/or reduced fertility, or is at risk of developing such a condition, comprising administering to the patient an effective amount of a compound of formula I as defined in claim 1.
- 25 4. A method of treatment of a patient in need of modulation of PPAR (for example $\text{PPAR}\alpha$) activity, comprising administering to the patient an effective amount of a compound of formula I as defined in claim 1.

5. A method of treatment of a patient in need of modulation of lipid or eicosanoid status or function, comprising administering to the patient an effective amount of a compound of formula I as defined in claim 1.
- 5 6. Use of a compound of formula I as defined in claim 1 in the manufacture of a medicament for treating a patient in need of modulation of PPAR (for example PPAR α) activity.
7. The method of claim 4 or use of claim 6 wherein the patient is in need of an
10 increase in PPAR activity and the compound is a PPAR agonist.
8. The method or use of claim 7 wherein the PPAR is PPAR α or PPAR γ .
9. The use of a compound of formula I as defined in claim 1 in the manufacture
15 of a medicament for the treatment of a patient in need of modulation of body mass or modulation of increase in body mass, and/or in need of modulation of plasma insulin, plasma glucose, plasma triglycerides, plasma cholesterol and/or leptin.
- 20 10. The method of claim 1 or use of claim 9 wherein the patient is in need of reduction of body mass or prevention of increase in body mass, and/or in need of reduction of plasma insulin, plasma glucose, plasma triglycerides, plasma cholesterol and/or leptin.
- 25 11. The use of a compound of formula I as defined in claim 1 in the manufacture of a medicament for the treatment of a patient who is overweight or obese and/or has diabetes, hyperlipidaemia, atherosclerosis, coronary heart disease, stroke, obstructive sleep apnoea, arthritis and/or reduced fertility, or is at risk of developing such a condition.

12. The use of a compound of formula I in the manufacture of a medicament for the treatment of a patient in need an antitumour agent or an antiinflammatory agent or of modulation of lipid or eicosanoid status or function, or of modulation of a lipid metabolising or binding entity activity.

5

13. The method of treatment or use of any one of claims 1 to 12 wherein the compound is or comprises a perfluorinated fatty acid or derivative thereof.

14. The method of treatment or use of any one of claims 1 to 12, wherein the
10 compound is or comprises a fluorinated carboxylic acid or perfluorinated carboxylic acid or pharmaceutically acceptable salt, ester or halide thereof.

15. The compound, method or use of claim 13 or 14 wherein the compound is perfluorooctanoic acid (PFOA) or a pharmaceutically acceptable salt, ester or
15 halide thereof, for example ammonium perfluorooctanoate (APFO).

16. The method or use of any one of claims 1 to 15 wherein the patient is human.

20 17. A screening method for identifying a drug-like compound or lead compound for the development of a drug-like compound in which (1) a mammal is exposed to a compound of formula I as defined in claim 1 or derivative thereof (2) the plasma insulin, glucose, cholesterol, triglyceride and/or leptin level of the mammal is measured, and/or bodyweight of the
25 mammal is measured, and/or lipid or eicosanoid status or function of the mammal is measured.

18. The method of claim 17 comprising the step of selecting a compound on exposure to which the plasma insulin, glucose, cholesterol, triglyceride and/or

leptin level of the mammal is changed or reduced, and/or bodyweight or bodyweight increase of the mammal is changed or reduced.

19. A screening method for identifying a drug-like compound or lead
5 compound for the development of a drug-like compound in which (1) a compound of formula I as defined in claim 1 or derivative thereof is exposed to a PPAR polypeptide (2) the binding of the compound to the PPAR polypeptide is measured or the change in the activity of the PPAR polypeptide is measured.

10 20. A screening method for identifying a drug-like compound or lead compound for the development of a drug-like compound in which (1) a compound of formula I as defined in claim 1 or derivative thereof is exposed to a lipid metabolising or binding entity, for example cyclooxygenase (for example cyclooxygenase I or cyclooxygenase II) or phospholipase A (for example
15 phospholipase A2) (2) the binding of the compound to the lipid metabolising or binding entity is measured or the change in the activity of the lipid metabolising or binding entity is measured.

21. A screening method for identifying a drug-like compound or lead
20 compound for the development of a drug-like compound in which (1) a cell is exposed to a compound of formula I as defined herein (for example a perfluorinated fatty acid) or derivative thereof (2) the phenotype (for example differentiation) and/or eicosanoid biosynthesis of the cell is measured.

25 22. The method of claim 21 further comprising the step of selecting a compound on exposure to which the phenotype, for example differentiation, of the cell is changed, and/or eicosanoid biosynthesis of the cell is changed, preferably reduced.

23. A compound identifiable or identified by a screening method according to any one of claims 17 to 22.

24. A compound identified or identifiable by a screening method of according
5 to any one of claims 17 to 22 for use in medicine.

25. The use of a compound identified or identifiable by a screening method according to any one of claims 17 to 22 in the manufacture of a medicament for the treatment of a patient in need of modulation of body mass or modulation of
10 increase in body mass, and/or in need of modulation of plasma insulin, plasma glucose, plasma triglycerides, plasma cholesterol and/or leptin.

26. Use of a compound identified or identifiable by a screening method according to any one of claims 17 to 22 in the manufacture of a medicament for
15 the treatment of a patient who is overweight or obese and/or has diabetes, hyperlipidaemia, atherosclerosis, coronary heart disease, stroke, obstructive sleep apnoea, arthritis and/or reduced fertility, or is at risk of developing such a condition.

20 27. The use of a compound identified or identifiable by a screening method according to any one of claims 17 to 22 in the manufacture of a medicament for treating a patient in need of modulation of PPAR (for example PPAR α) activity, or in need of an antitumour agent or an antiinflammatory agent, or in need of modulation of lipid or eicosanoid status or function, or in need of
25 modulation of lipid metabolising or binding entity activity.

28. The use of claim 27 wherein the patient is in need of an increase in PPAR activity and the compound is a PPAR (for example a PPAR α) agonist.

29. A method of treatment of a patient in need of modulation of body mass or modulation of increase in body mass, and/or in need of modulation of plasma insulin, plasma glucose, plasma triglycerides, plasma cholesterol and/or leptin, comprising administering to the patient an effective amount of a compound
5 identified or identifiable by the screening method of any one of claims 17 to 22.

30. A method of treatment of a patient who is overweight or obese and/or has diabetes, hyperlipidaemia, atherosclerosis, coronary heart disease, stroke, obstructive sleep apnoea, arthritis and/or reduced fertility, or is at risk of
10 developing such a condition, comprising administering to the patient an effective amount of a compound identified or identifiable by the screening method of any one of claims 17 to 22.

31. A method of treatment of a patient in need of modulation of PPAR (for
15 example PPAR α) activity, or in need of an antitumour agent or an antiinflammatory agent, or in need of modulation of lipid or eicosanoid status or function, or of a lipid metabolising or binding entity activity, comprising administering to the patient an effective amount of a compound identified or identifiable by the screening method of any one of claims 17 to 22.

20

32. A compound identified or identifiable by a screening method of any one of claims 17 to 22 for use in the manufacture of a composition for use as a food supplement or a food additive.

25 33. A food product comprising a foodstuff and a compound of formula I as defined in claim 1 or a compound identified or identifiable by a screening method of any one of claims 17 to 22, wherein the food is not laboratory rodent feed.

34. A kit of parts of screening system comprising (1) a library of compounds each of formula I as herein defined or a derivative thereof, and (2) a PPAR polypeptide or polynucleotide encoding a PPAR polypeptide, and/or a test mammal.

5

35. A kit of parts of screening system comprising (1) a library of compounds each of formula I as herein defined or a derivative thereof, and (2) a lipid metabolising or binding entity (for example COXI or COXII or phospholipase A2 or lipoxygenase) or polynucleotide encoding a lipid metabolising or binding entity.

10

36. Any novel compound, use, kit, system or method as herein disclosed.

Figure 1

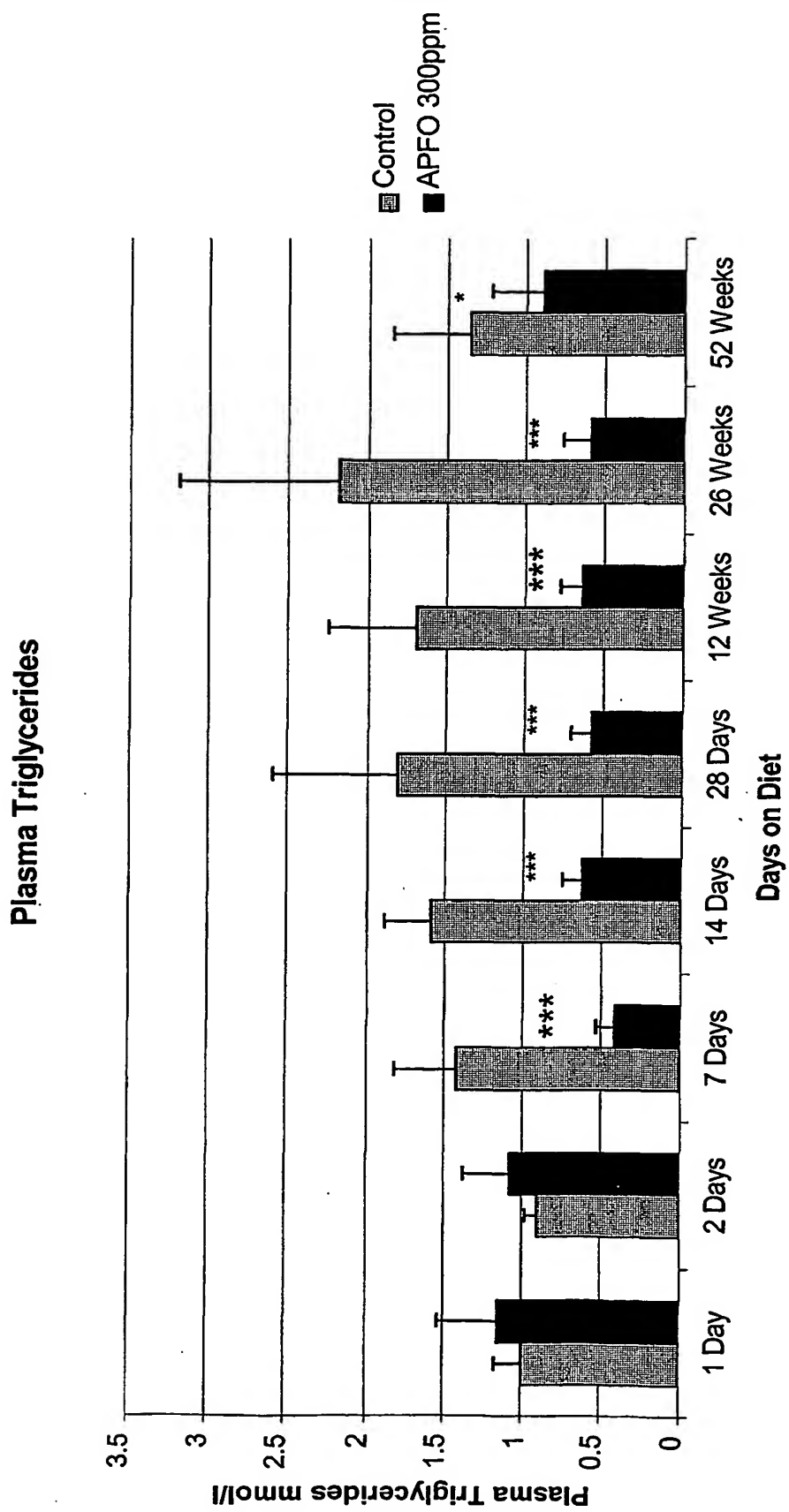


Figure 2

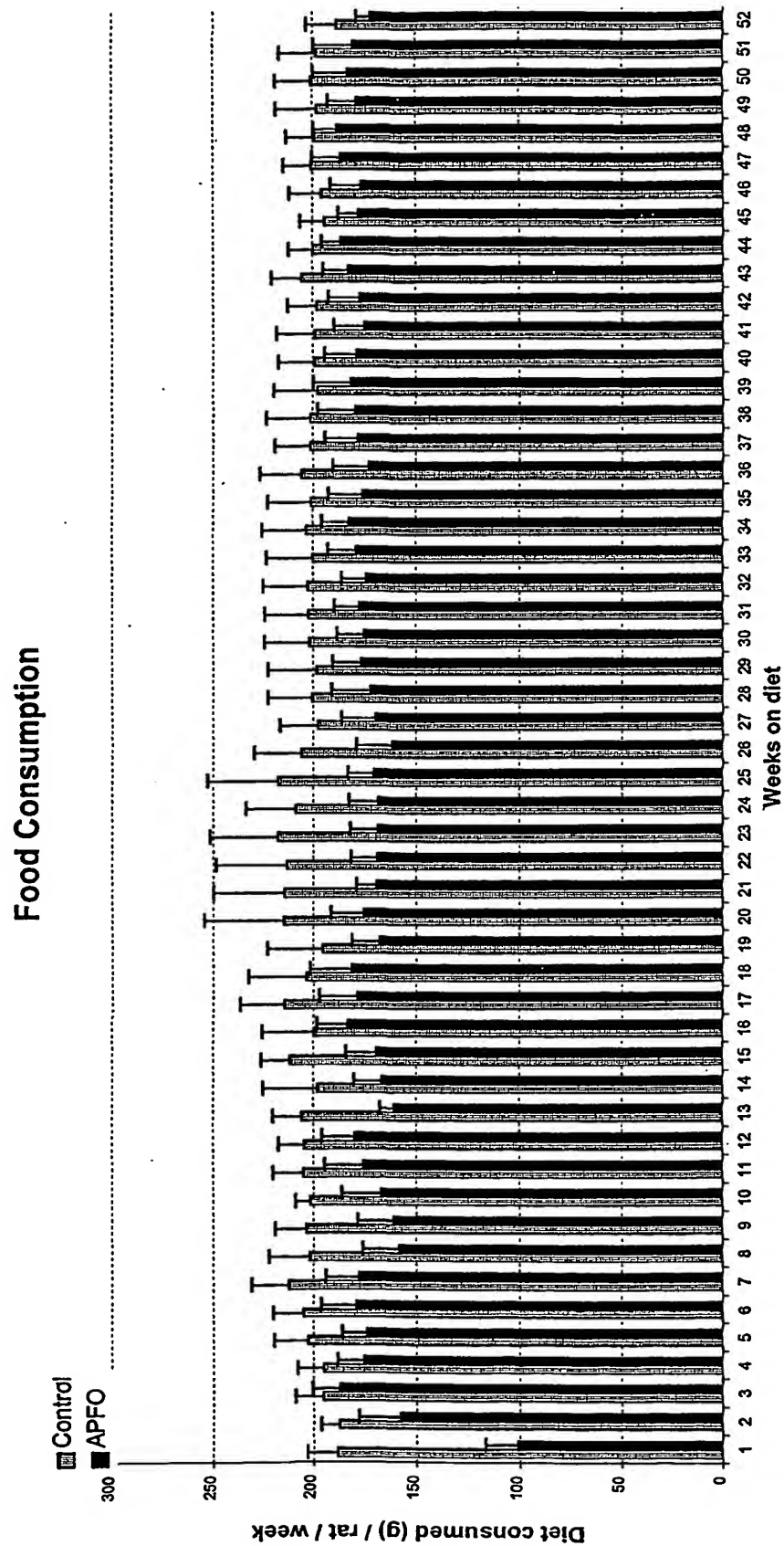


Figure 3

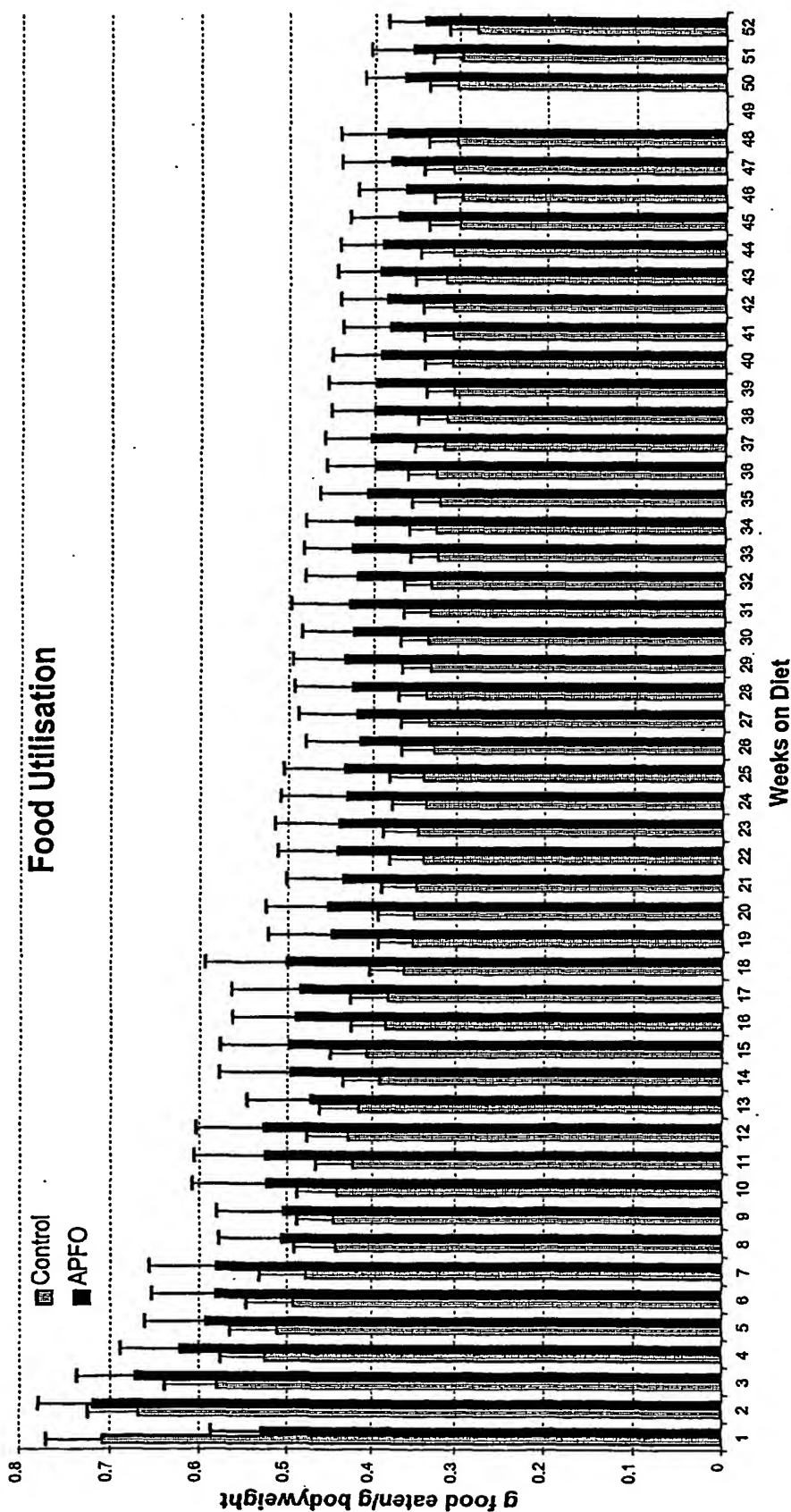


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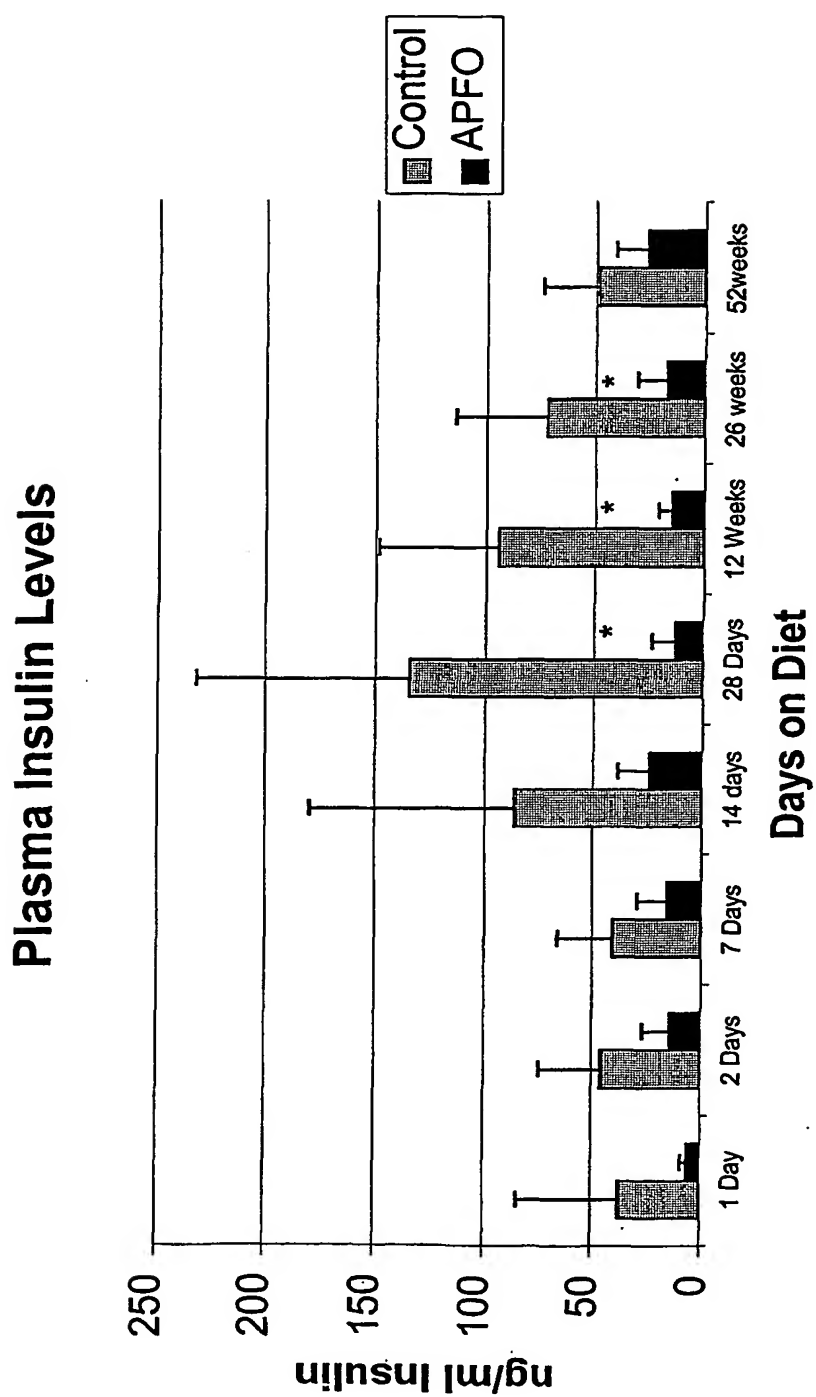


Figure 5

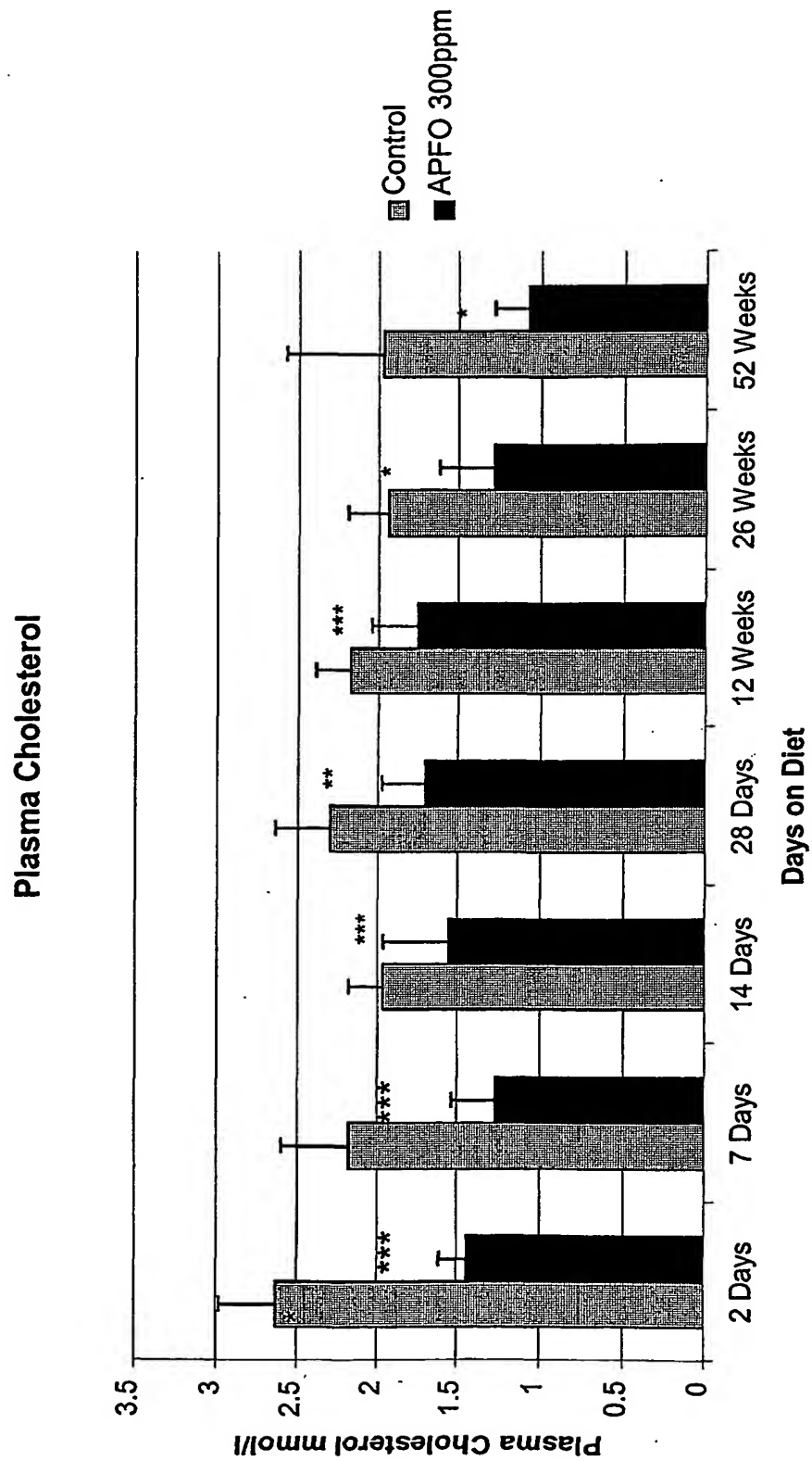


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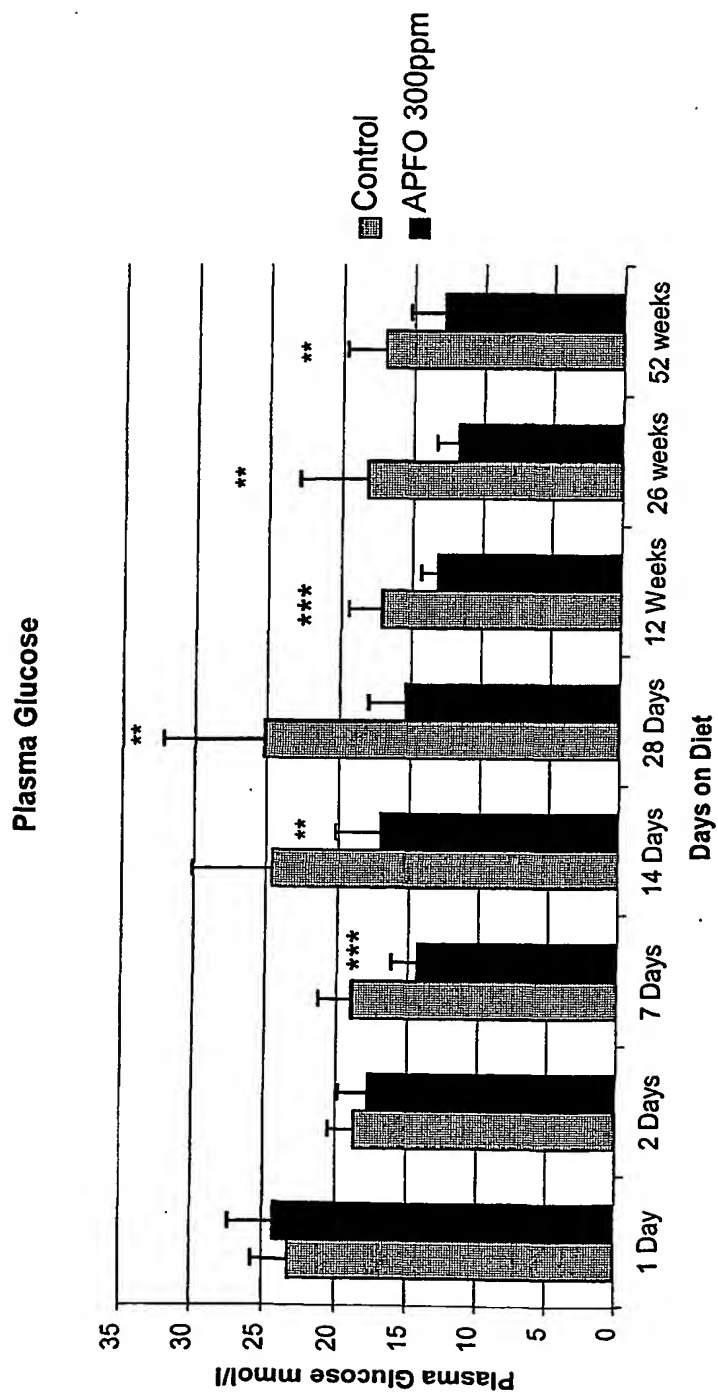


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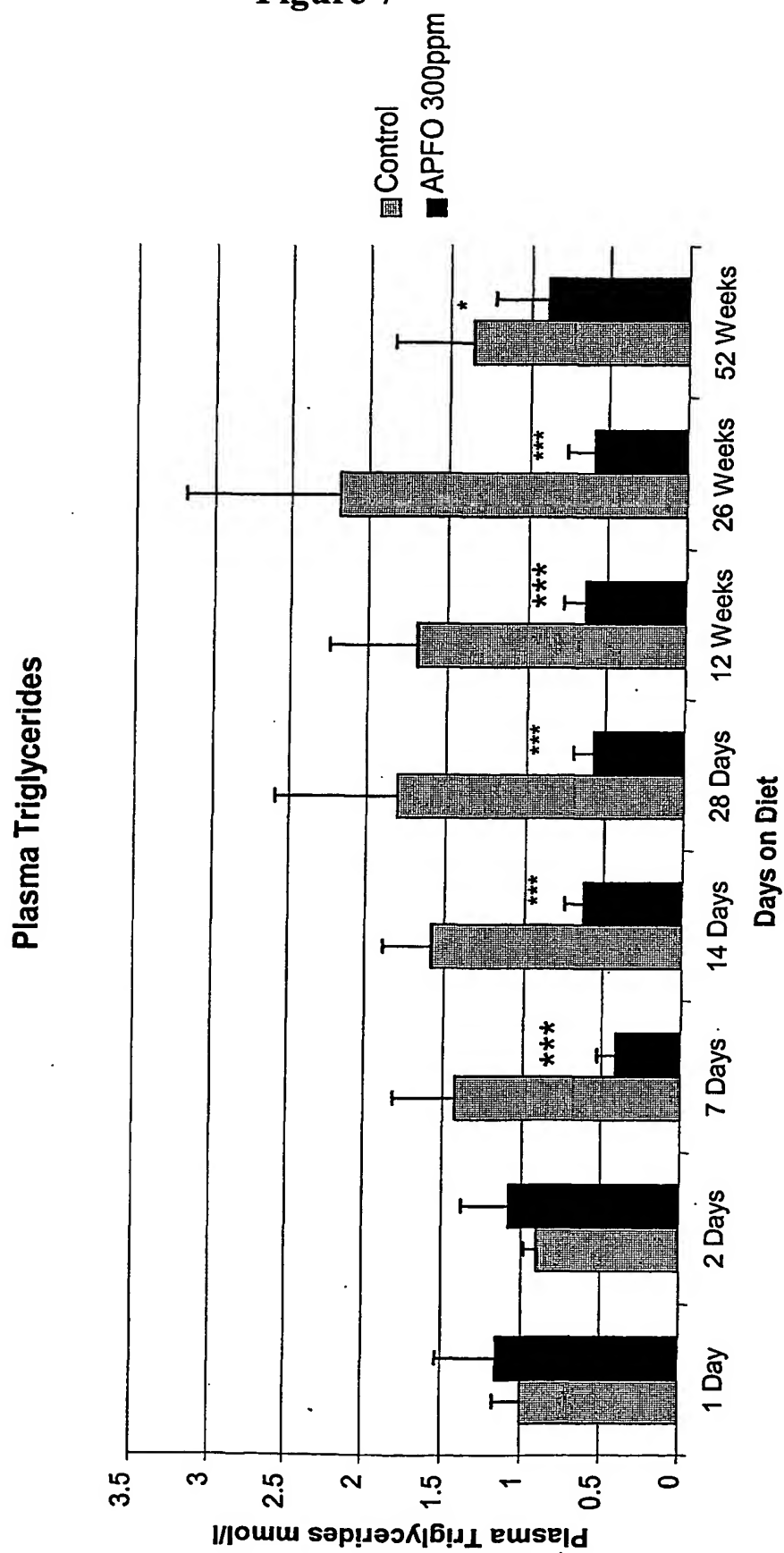
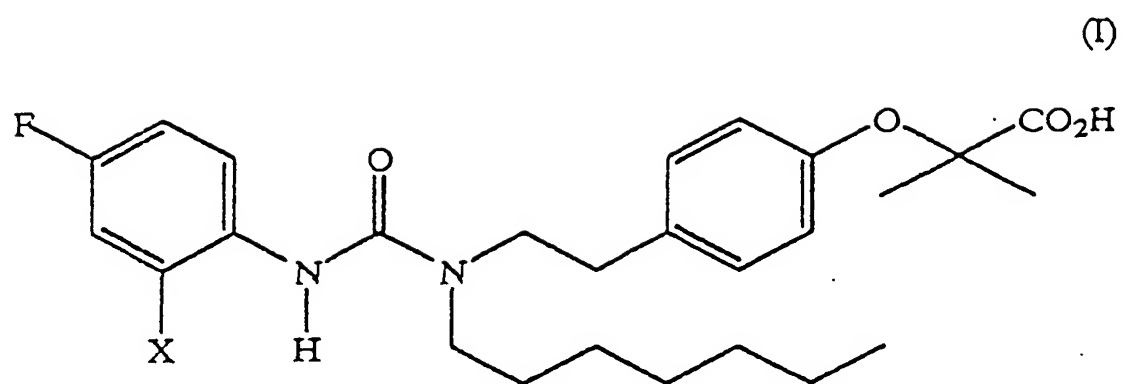


Figure 8



wherein X represents hydrogen or fluorine.

Figure 9

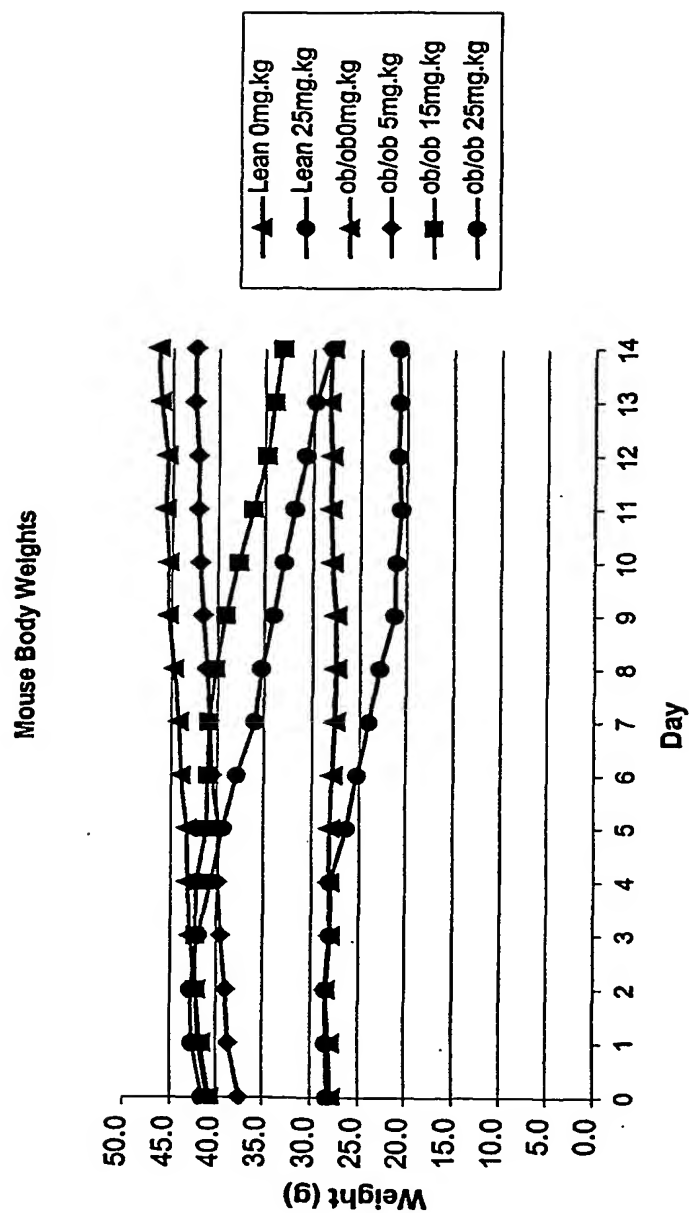


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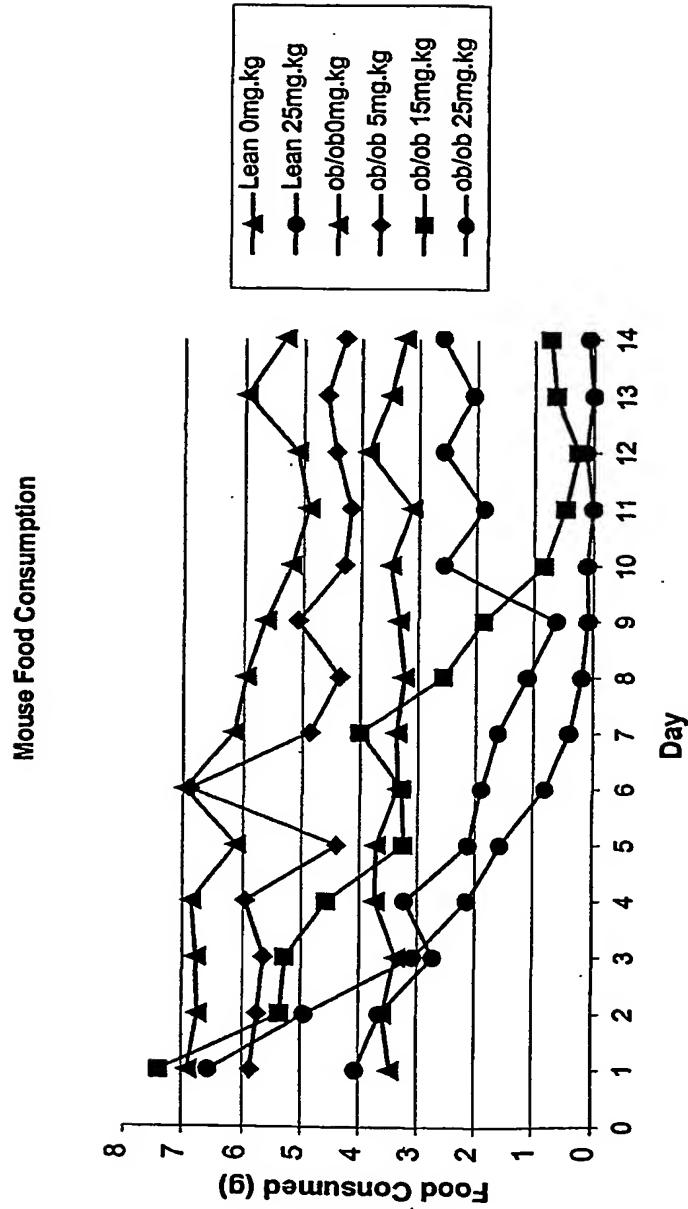


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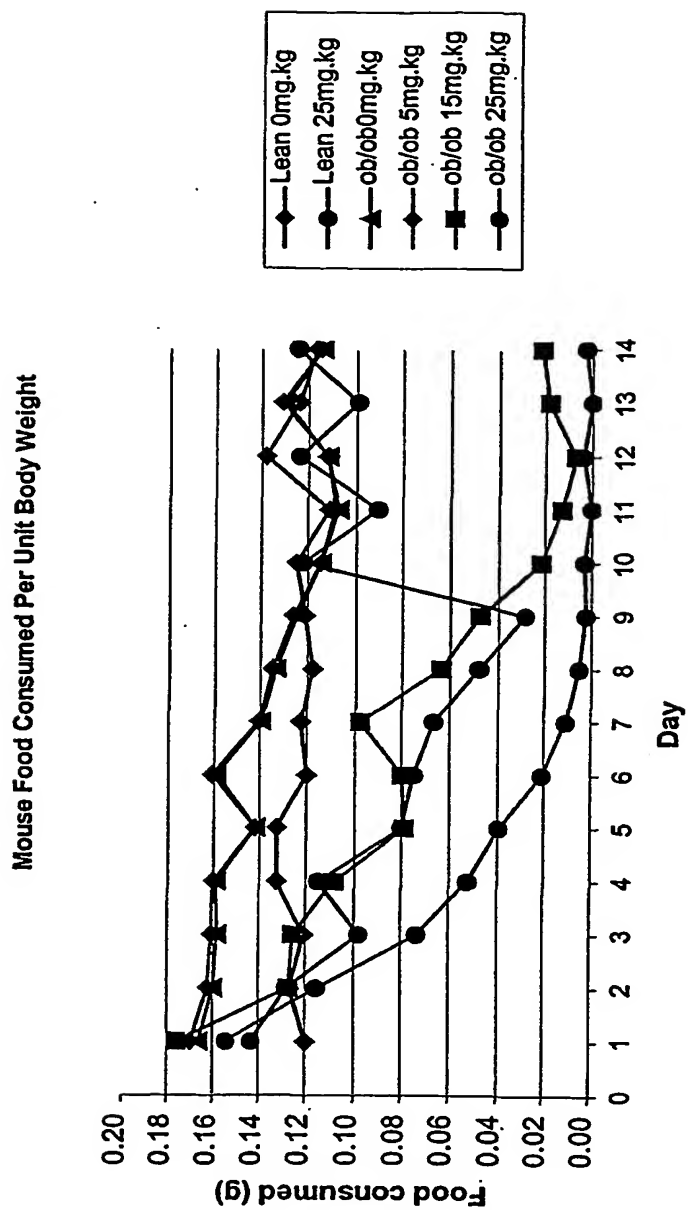


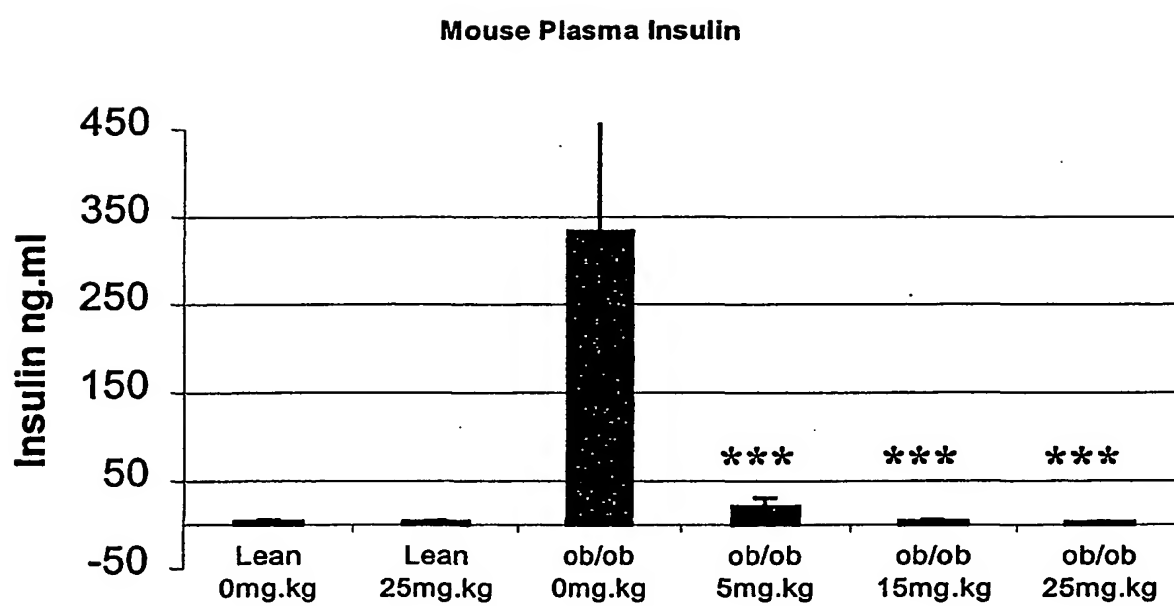
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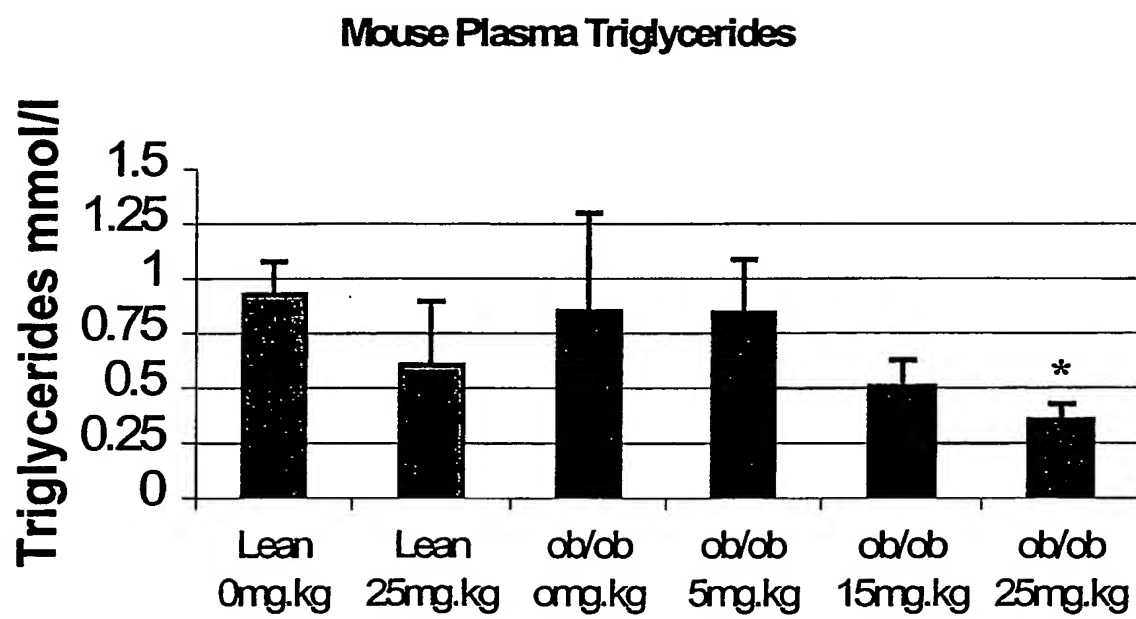
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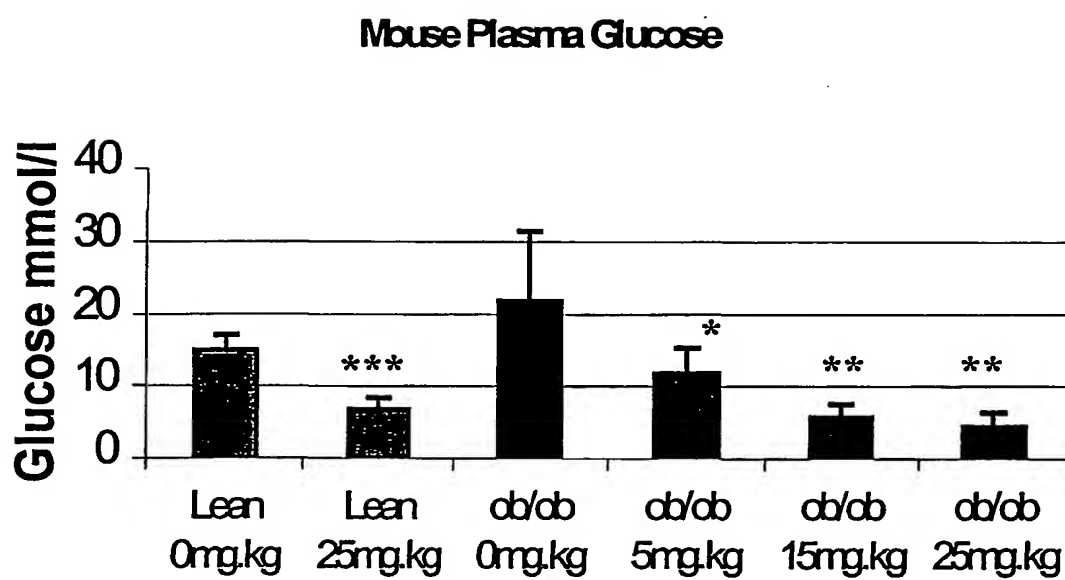
Figure 14

Figure 15

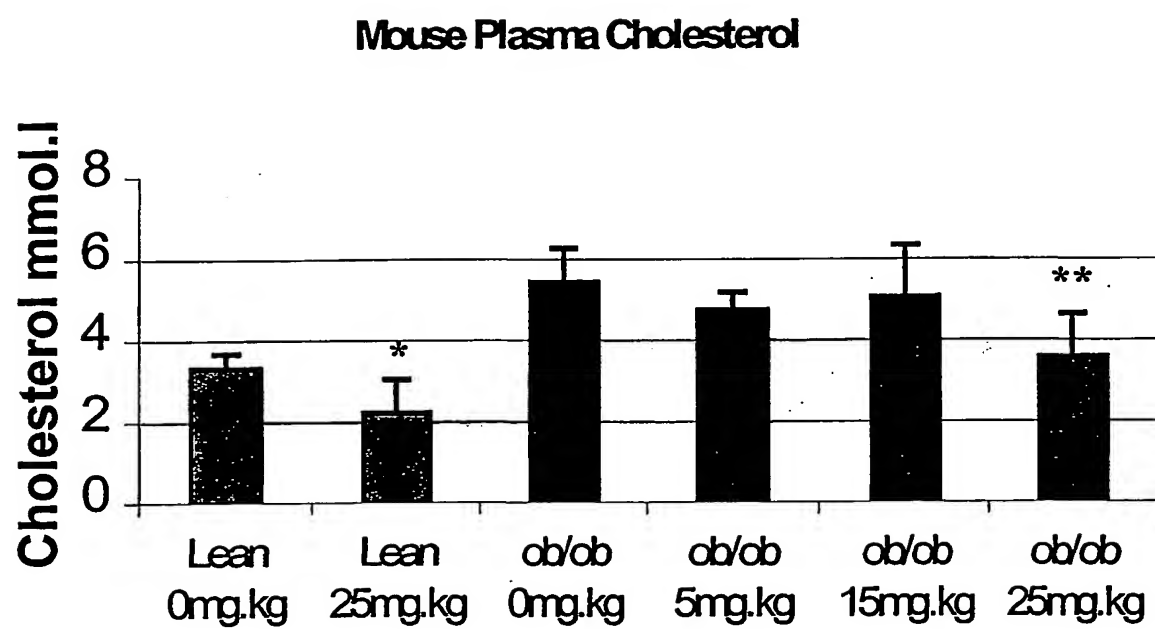


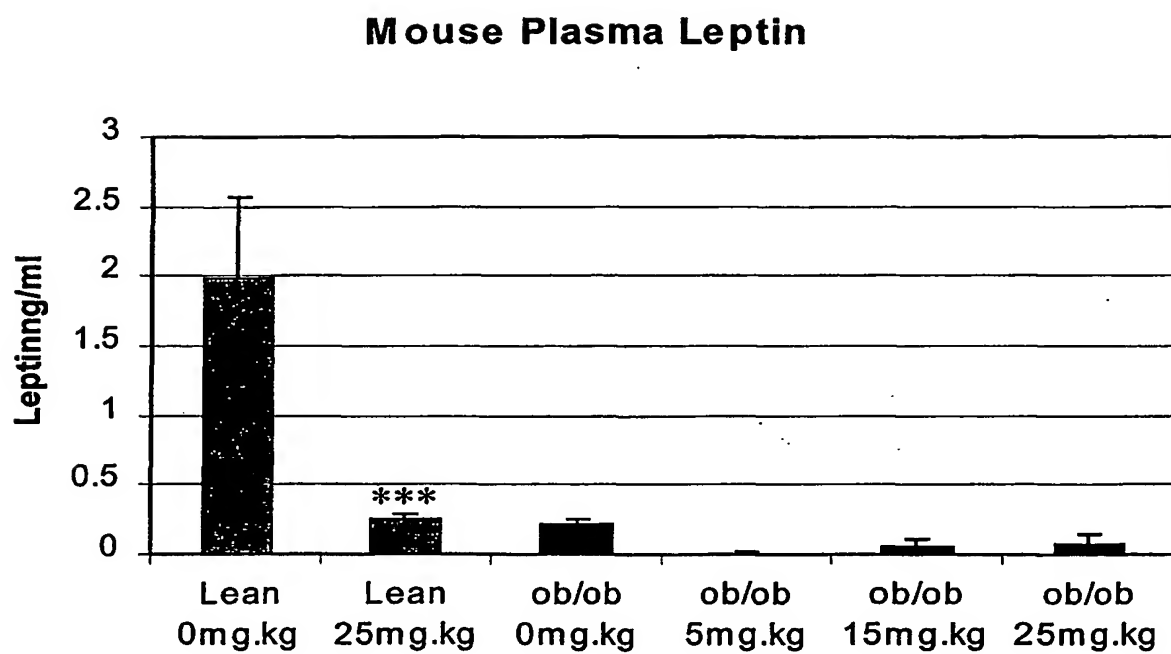
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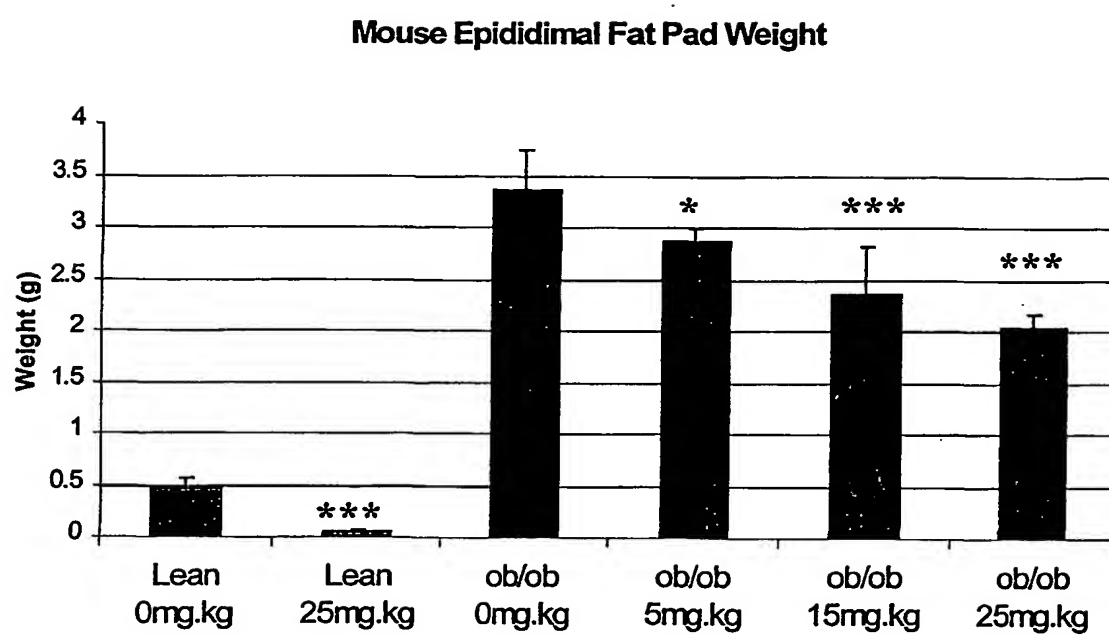
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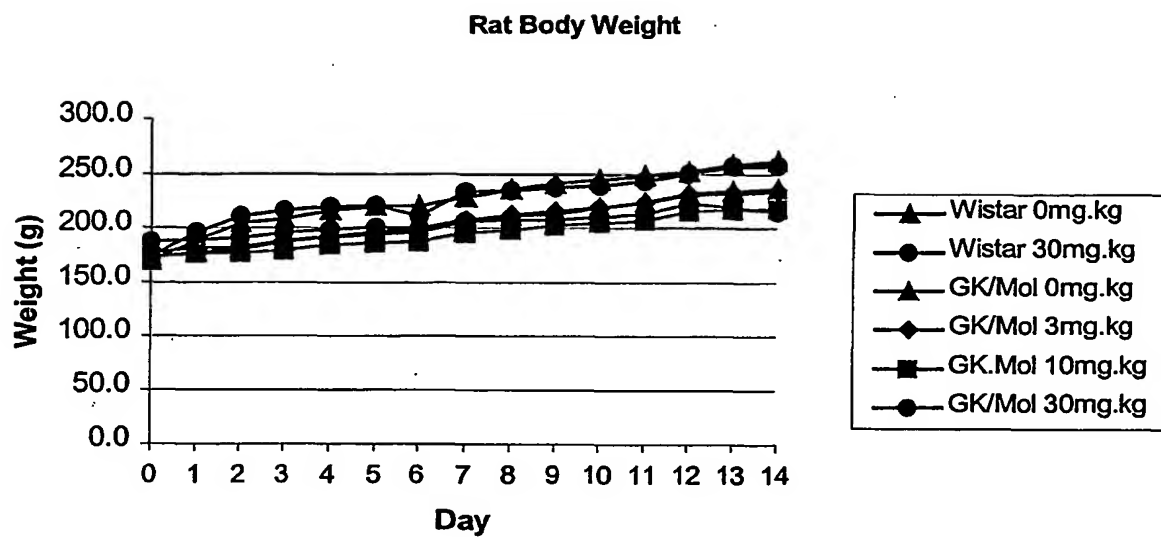
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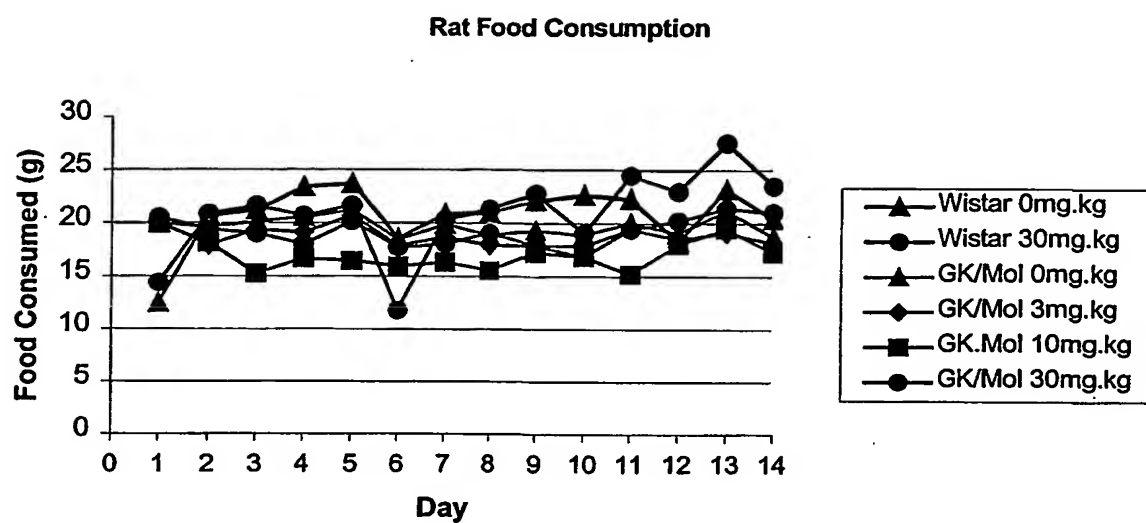
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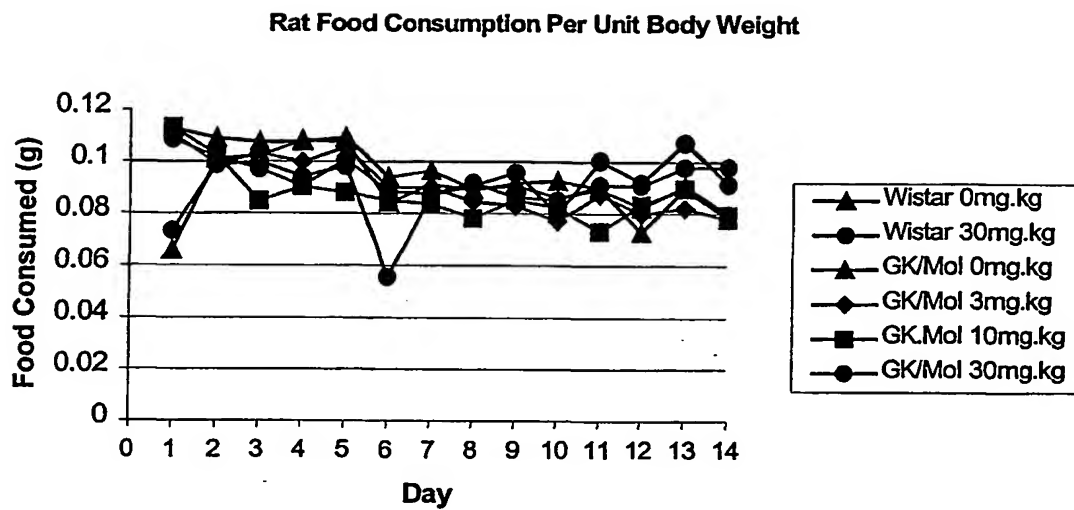
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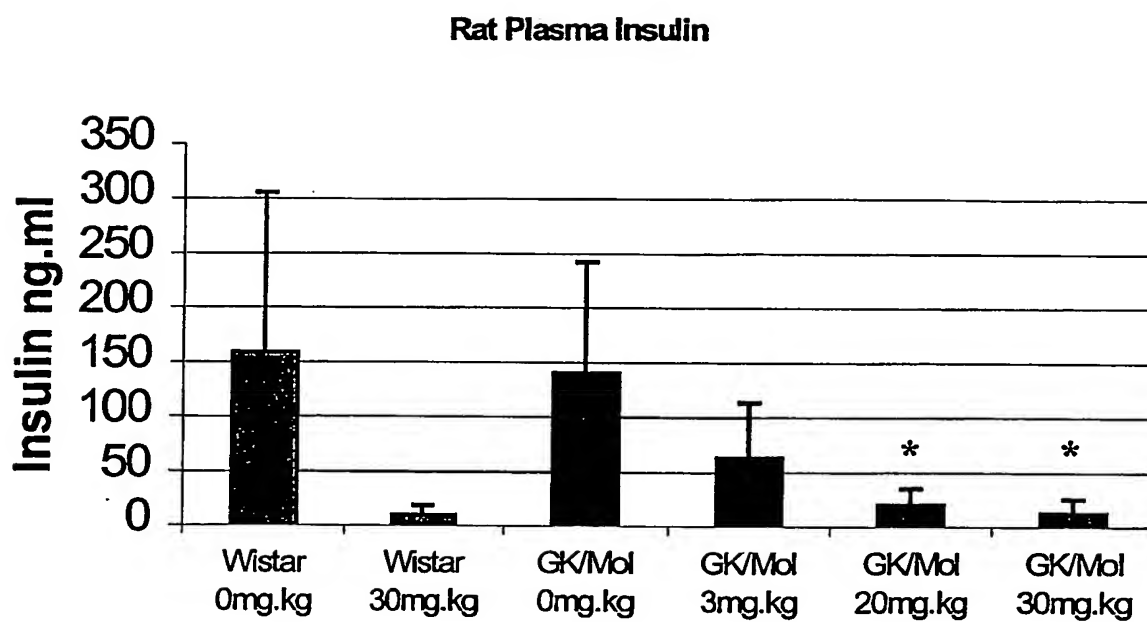
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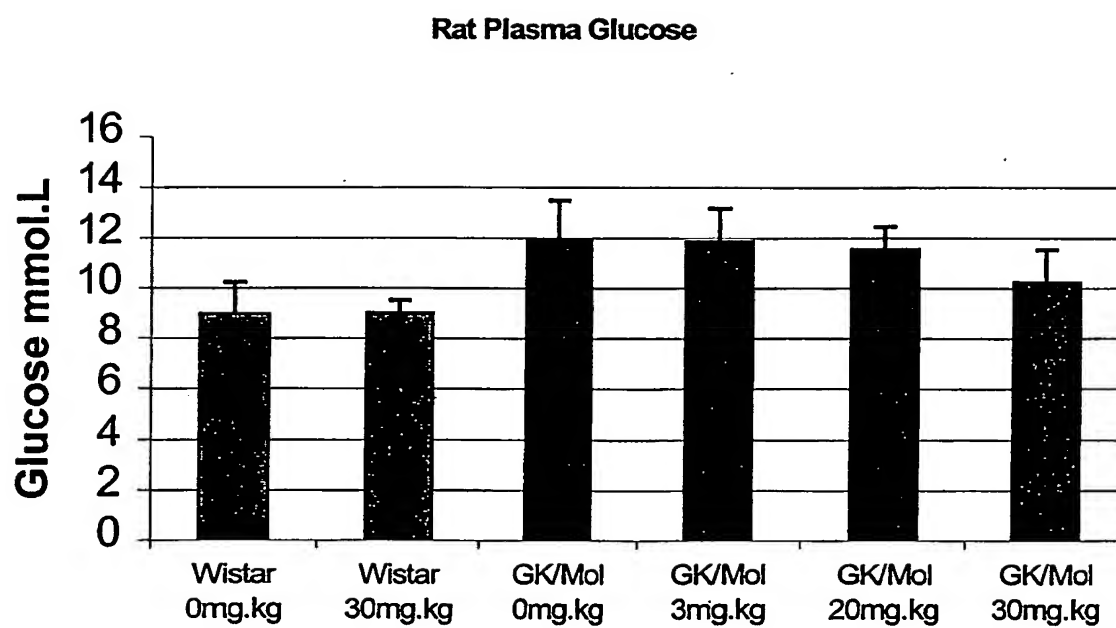
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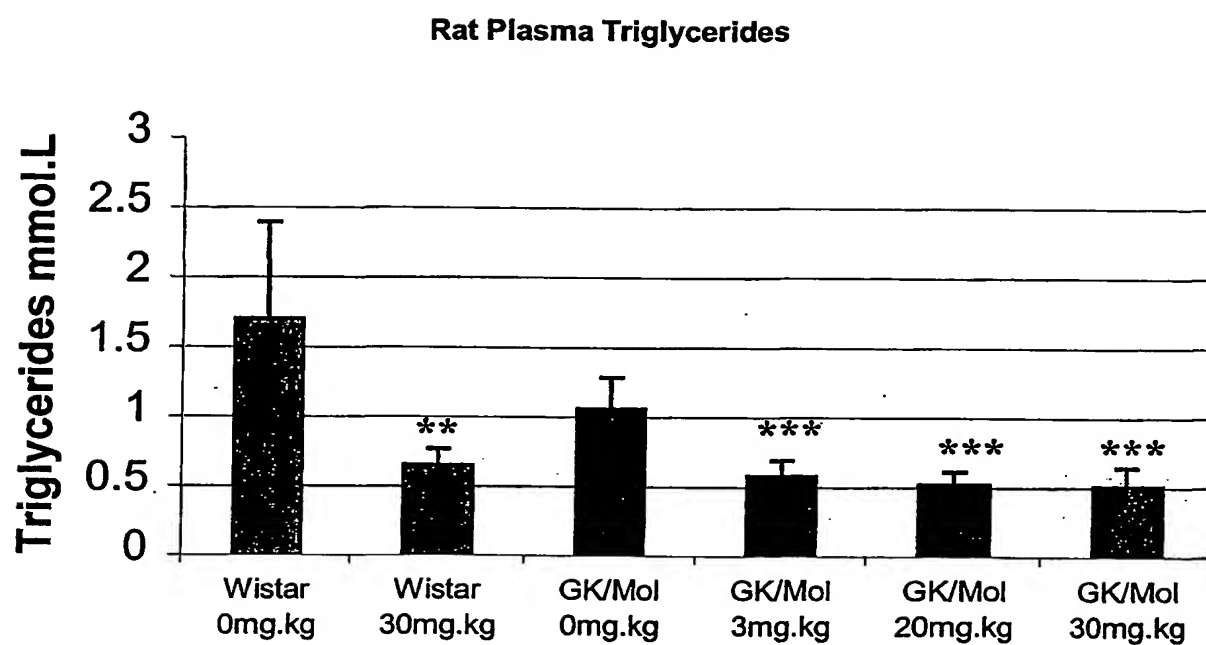
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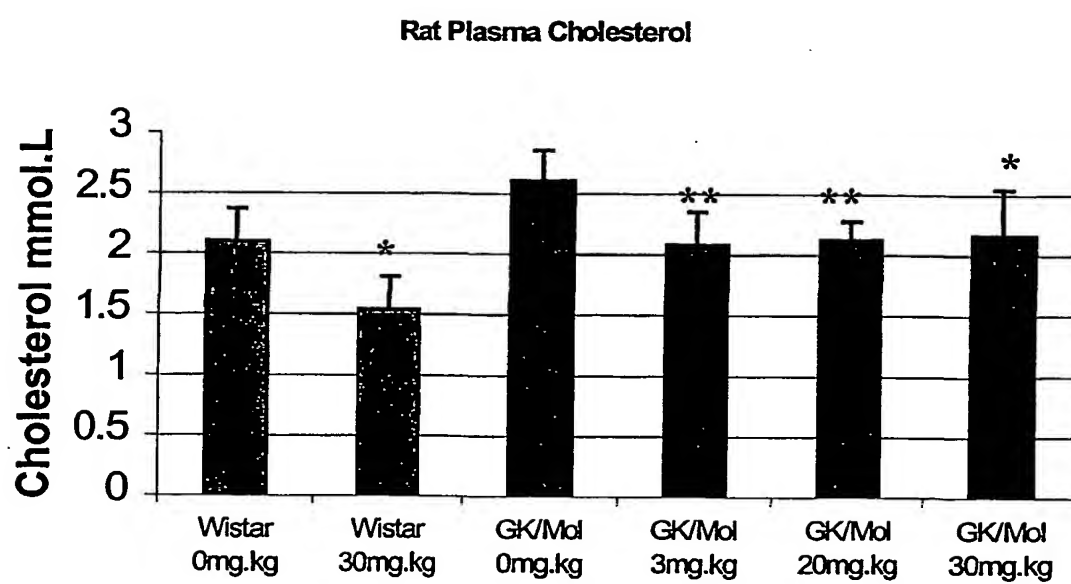
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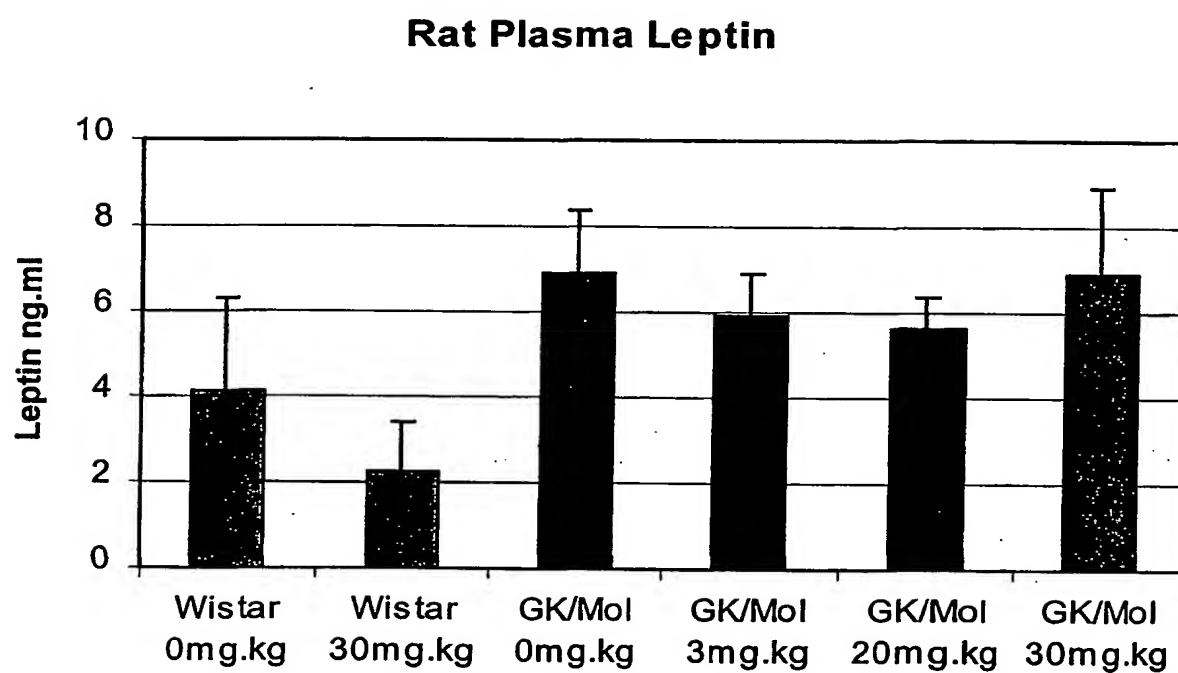
Figure 25

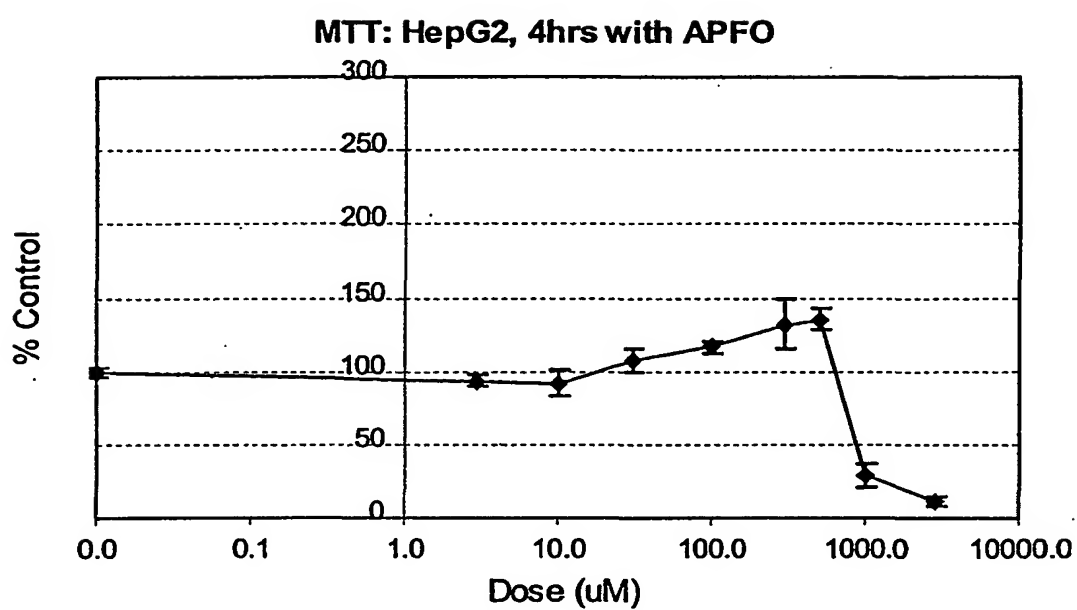
Figure 26**Part 1 of 3**

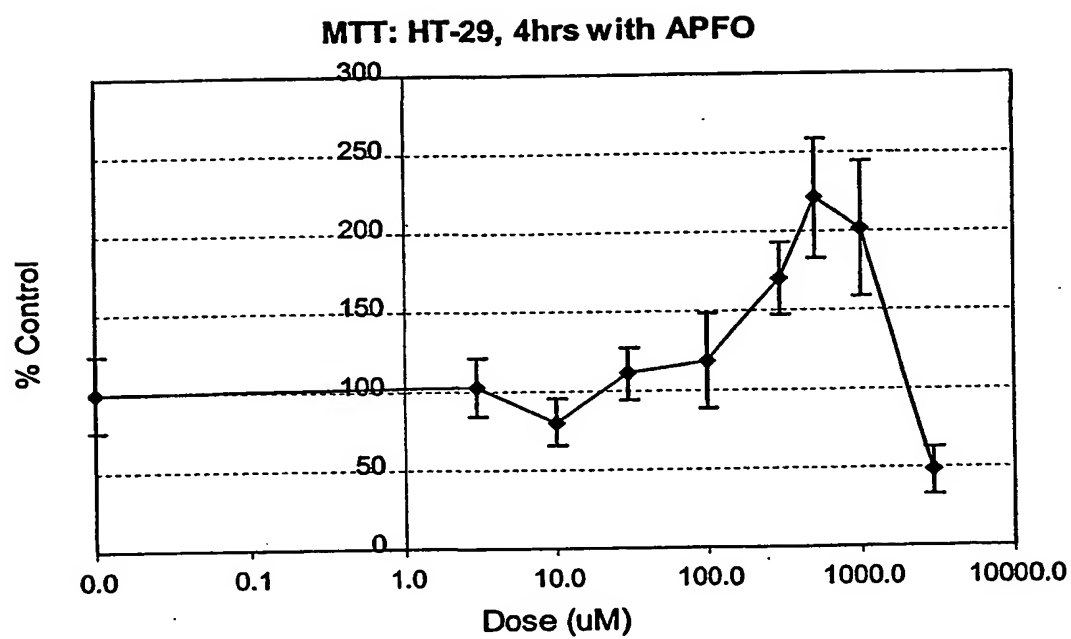
Figure 26**Part 2 of 3**

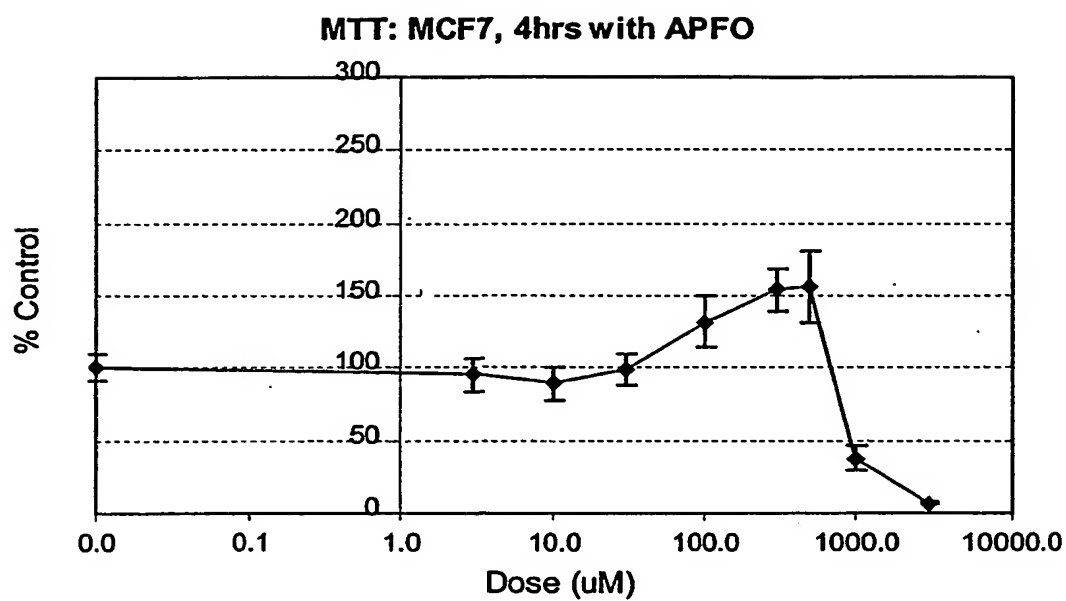
Figure 26**Part 3 of 3**

Figure 27

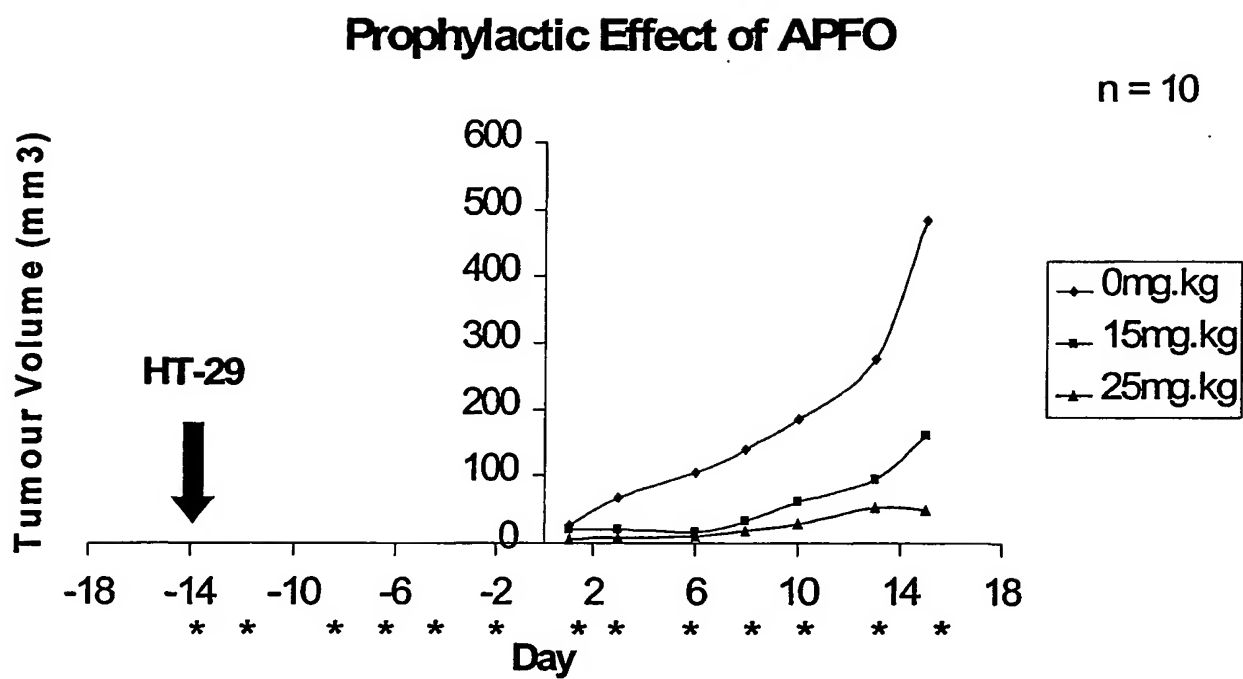


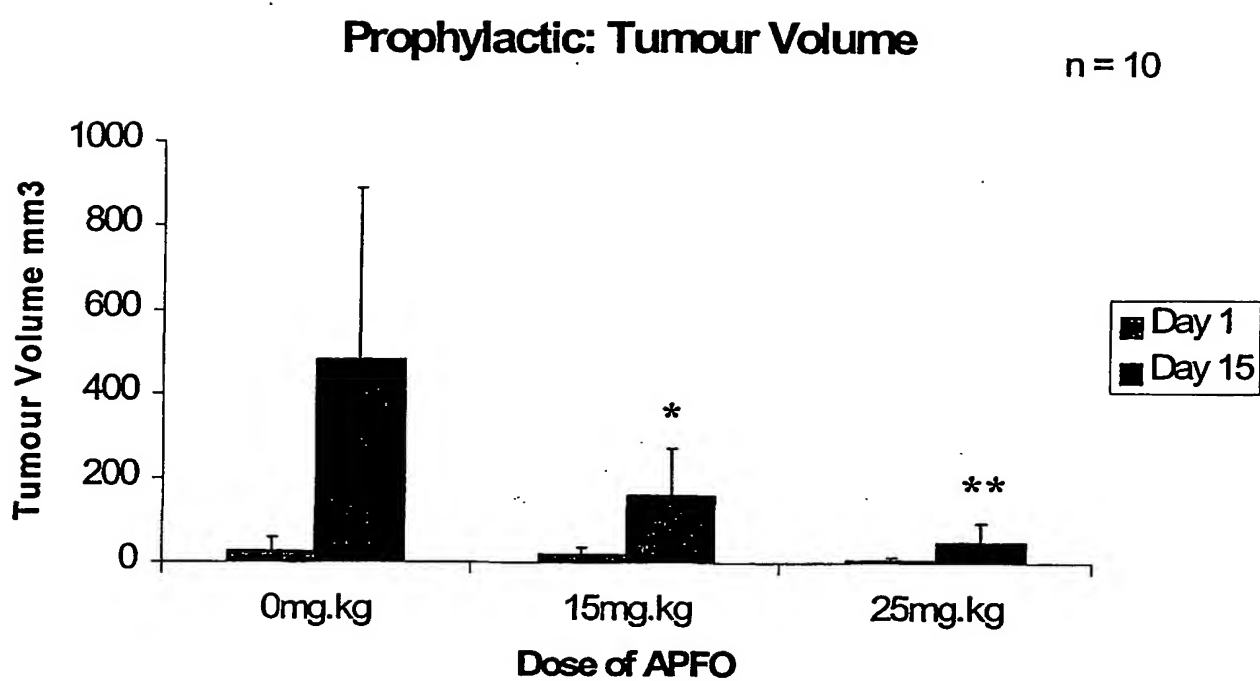
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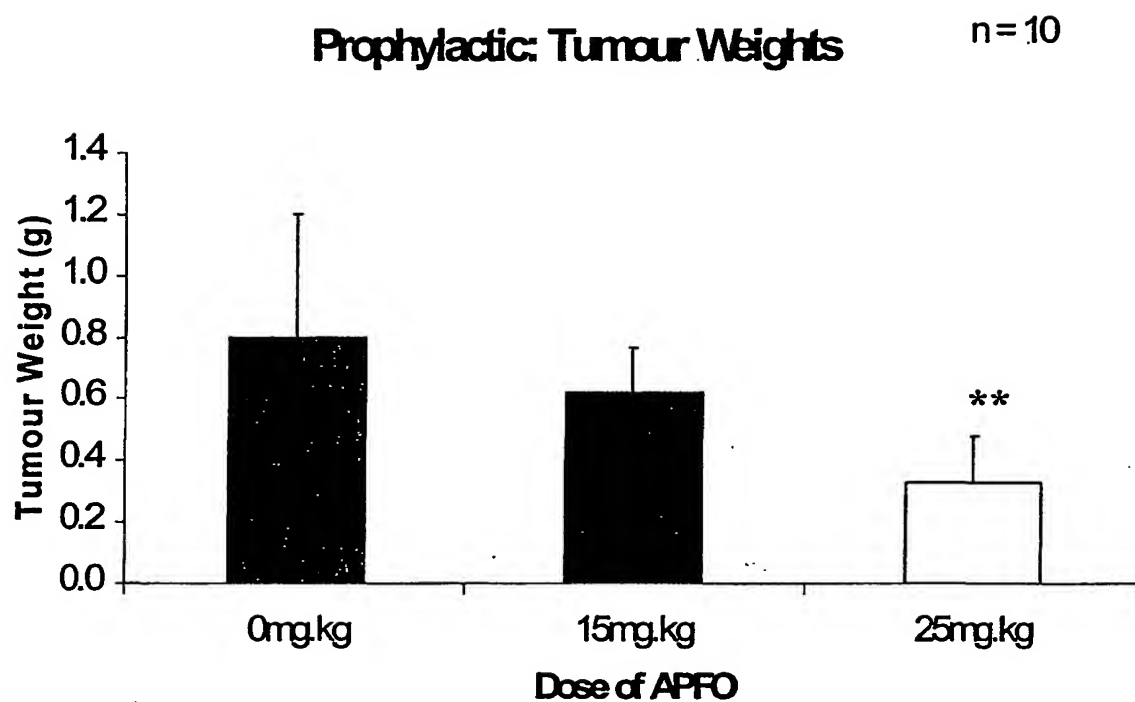
Figure 29

Figure 30

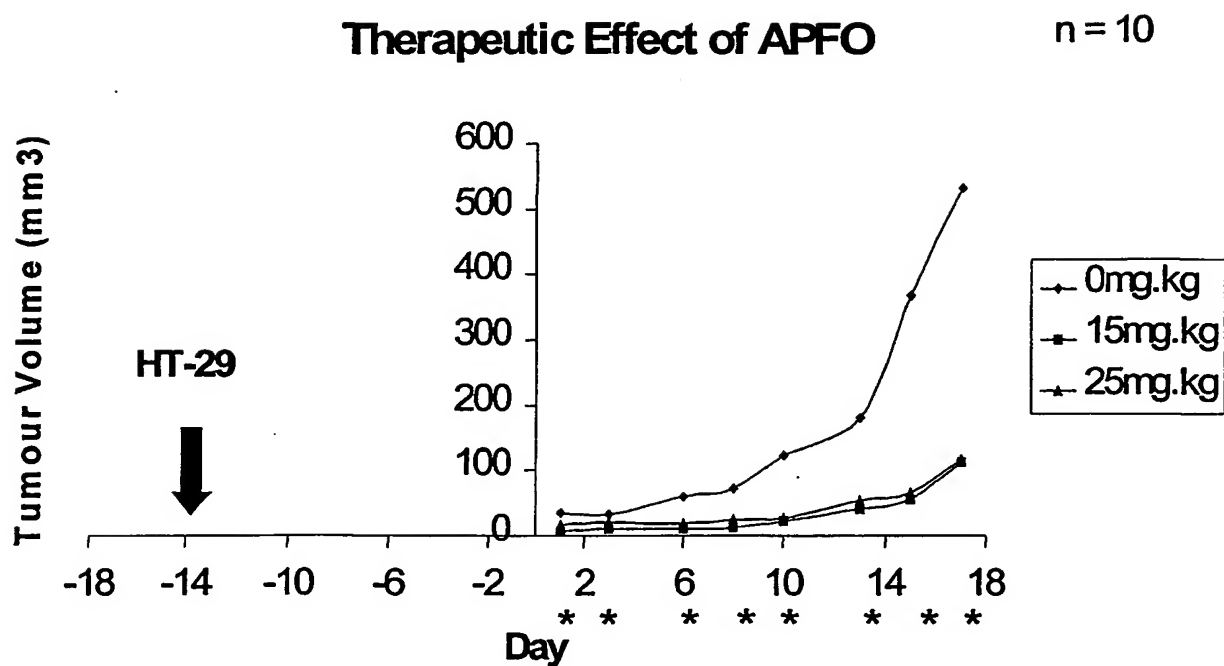


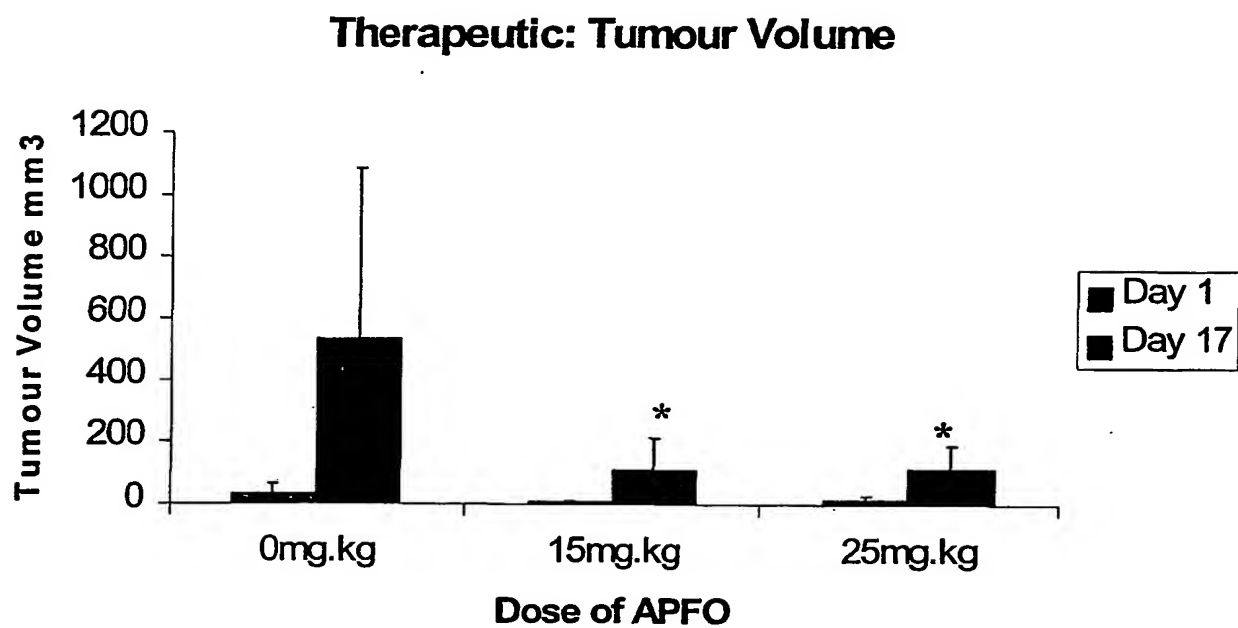
Figure 31

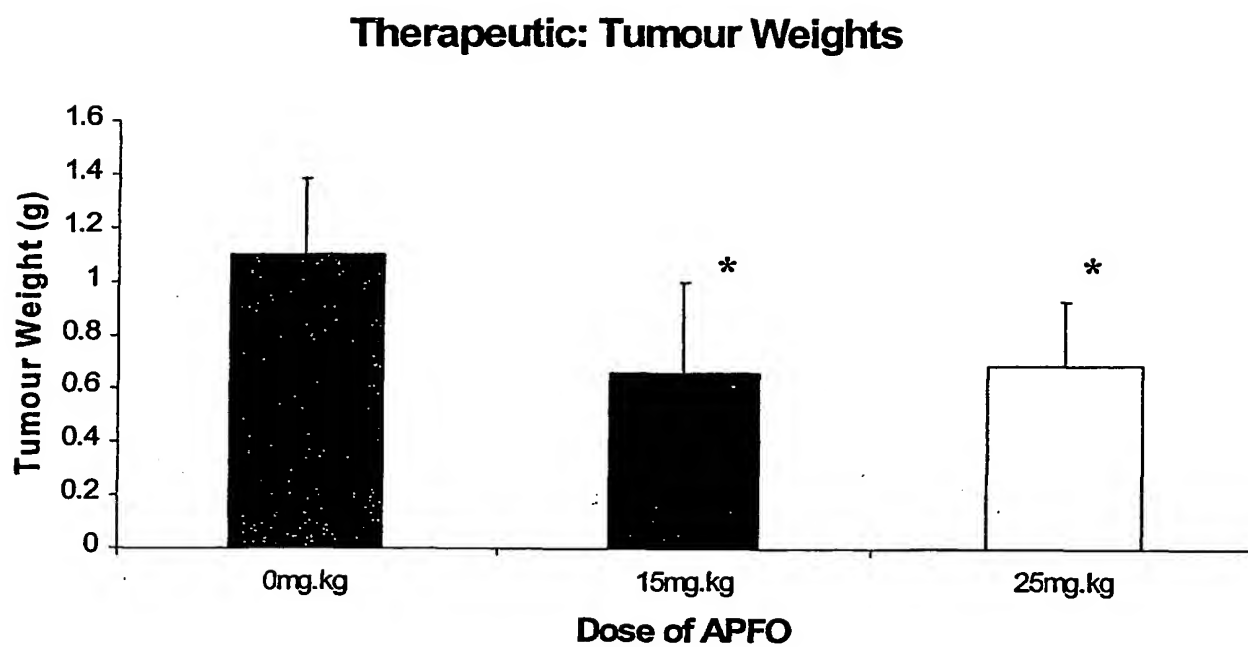
Figure 32

Figure 33

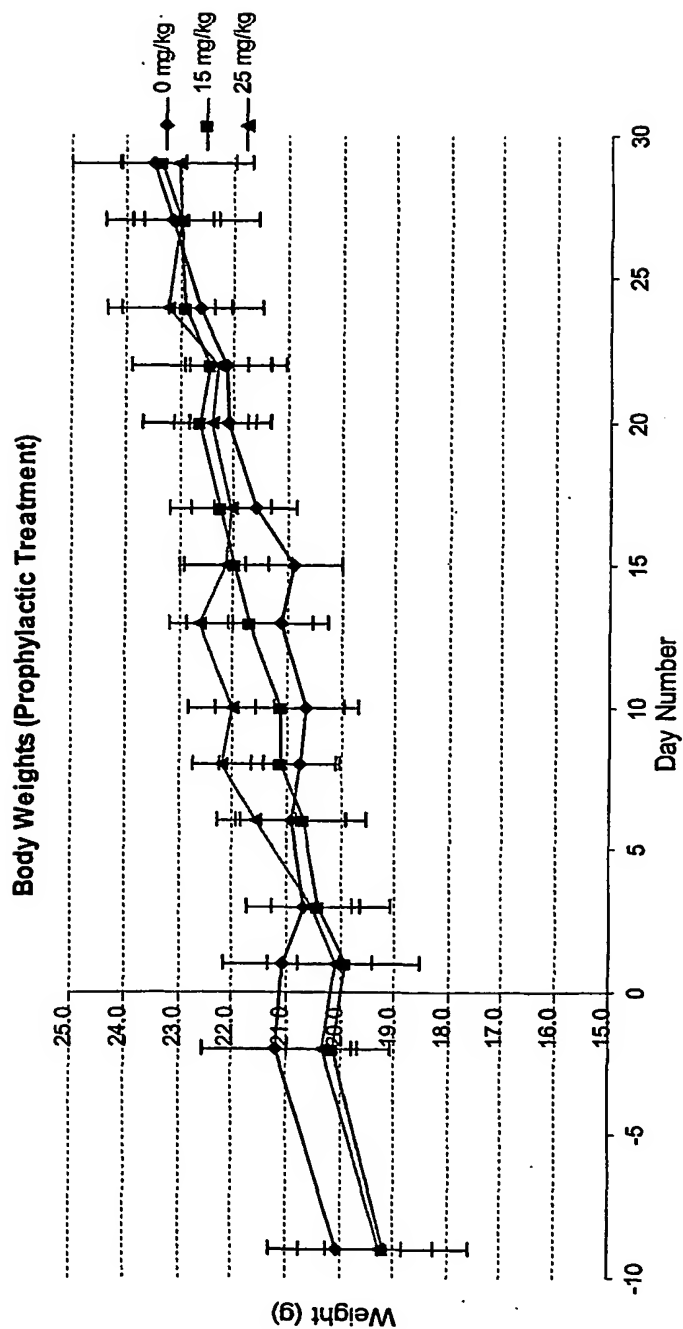


Figure 34

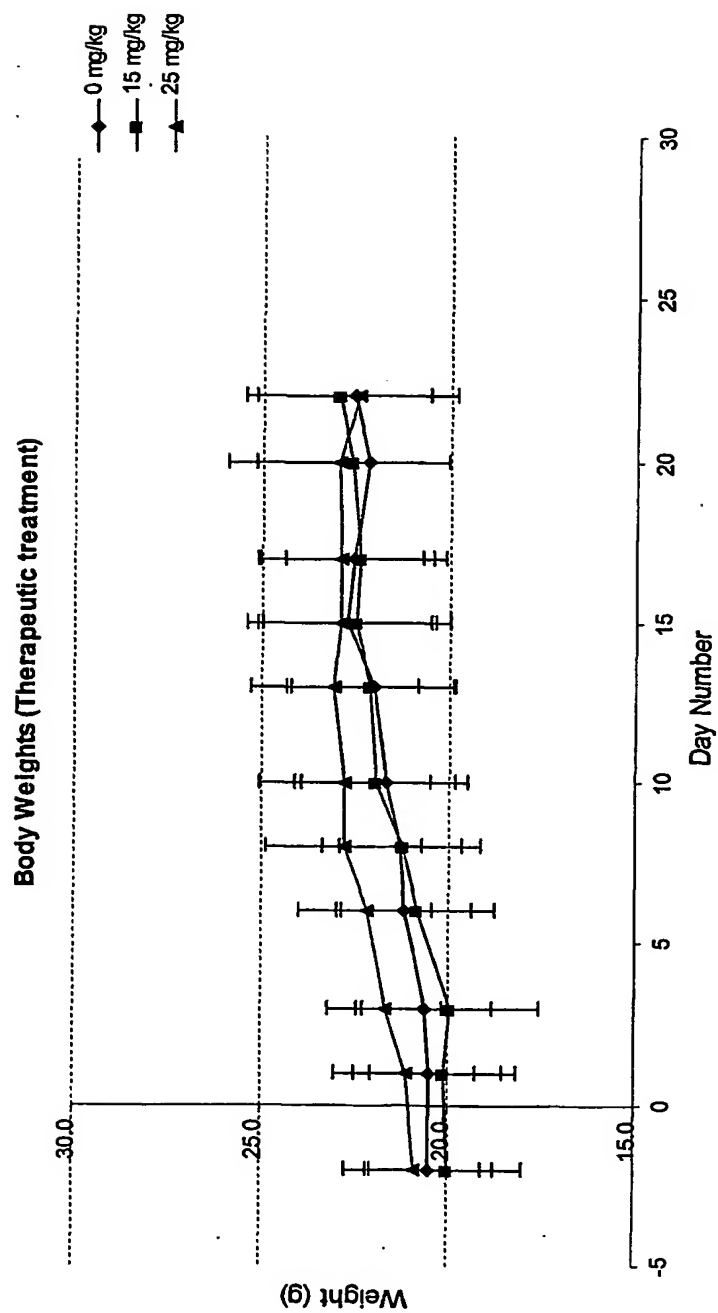


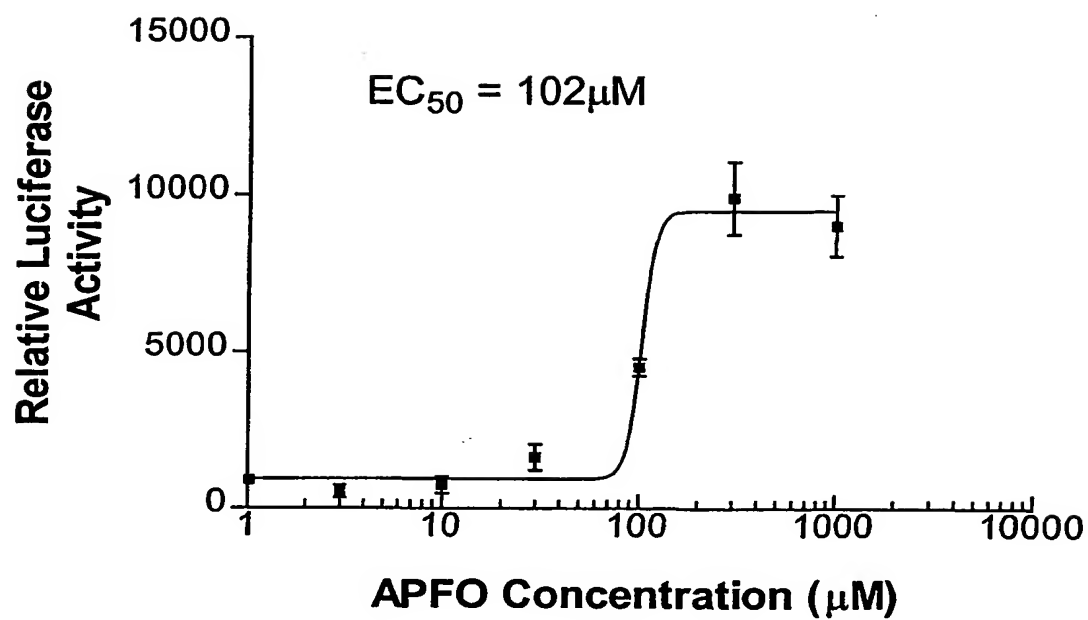
Figure 35

Figure 36